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Sixth Meeting of the European Association for Cancer Research
Union Internationale Contre le Cancer Conference on Clinical Oncology
Symposium on Herpesviruses as Oncogenic Agents

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1983.

Phase II Study of High-Dose Methotrexate in the Treatment of Patients With Non-Small Cell Carcinoma of the Lung: An Eastern Cooperative Oncology Group Study[1]

David S. Ettinger,* Kenneth E. Stanley, and J. Scott Nystrom[2]

SUMMARY

Twenty-eight patients with inoperable non-small cell carcinoma of the lung were treated with high-dose methotrexate with citrovorum factor rescue. One patient (4%) responded with a partial regression of the tumor. Three patients had severe or life-threatening complications: acute renal failure, acute shortness of breath, and bone marrow suppression, respectively. There is 98% confidence that the true response rate for this therapy is < 20%. The results of this study demonstrate that high-dose methotrexate with citrovorum factor rescue, in the schedule utilized, is not effective in the treatment of patients with non-small cell carcinoma of the lung.

[Cancer Treat Rep 64:1017–1021, 1980]

Previous trials are conflicting as to the activity of high-dose (HD) methotrexate (MTX) with citrovorum factor (CF) rescue in the treatment of non-small cell carcinoma of the lung (1–4). Moreover, only a small number of patients have been studied. The present study was designed to determine the therapeutic effectiveness of HDMTX-CF rescue in patients with inoperable non-small cell carcinoma of the lung.

MATERIALS AND METHODS

Between May 1976 and June 1978, 34 patients with histologically confirmed, inoperable, measurable non-small cell carcinoma of the lung were entered in the study. Patients were further required to have an initial Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 3 or better, adequate bone marrow function (wbc count of > 4000/mm^3 and platelet count of > 100,000/mm^3), adequate hepatic function (bilirubin < 1.5 mg/100 ml and SGOT < 60 IU), adequate renal function (creatinine < 1.5 mg/100 ml and creatinine clearance > 70 ml/minute), evidence of progressive disease following radiotherapy if irradiation was given prior to study entry, and no previous treatment with cytotoxic drugs. Data from 28 patients were analyzed. Five patients initially entered in the study were dropped for the following reasons: refusal of treatment (two patients), drop to an ECOG PS of 4 before treatment (one), death before beginning of therapy (one), and referral by the physician for radiotherapy (one). One patient was considered ineligible because there was no objective evidence of progressive disease after radiotherapy.

All patients were adequately hydrated prior to the MTX infusion. With the start of the infusion, hydration was continued with at least 2500 ml/m^2/24 hours iv and then orally until completion of the CF rescue. To maintain a urine pH > 7.0, polycitra or sodium bicarbonate was given to the patient 24 hours before the MTX infusion and for 72 hours

[1]Received Nov. 6, 1979; revised Feb 19, 1980; accepted Mar 5, 1980.

[2]This study was conducted by the Eastern Cooperative Oncology Group (Paul P. Carbone, chairman; CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: Johns Hopkins Oncology Center, Baltimore, MD (*D. S. Ettinger*; CA-16116), Sidney Farber Cancer Institute and Harvard University, Boston, MA (*K. E. Stanley*; CA-23318), and Yale School of Medicine, New Haven, CT (*J. S. Nystrom*; CA-15485). Other participating institutions include: Harbor General Hospital, Torrance, CA (CA-21091); Northwestern University Medical Center, Chicago, IL (CA-17145); and University of Ottawa, Ontario, Canada.

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following completion of the infusion. On Day 1 of the drug cycle, the patient received MTX at a dose of 1500 mg/m² iv over 6 hours, followed in 2 hours by the CF rescue. The dose of MTX was escalated at 2-week intervals by an additional 1500 mg/m² iv until a dose of 6000 mg/m² was reached. Thereafter, the MTX dose was escalated at 3-week intervals until 7500 mg/m² was reached, at which time all subsequent doses were to be given every 3 weeks until progression of disease. Two hours after completion of the MTX infusion, 5–20 mg of CF was administered iv every 3 hours × 8, then orally every 6 hours × 8. The amount of CF given was based on the body surface area of the patient (5 mg for < 0.5 m²; 10 mg for 0.5–1.0 m²; 15 mg for 1.0–2.0 m²; and 20 mg for > 2.0 m²). The regimen utilized in the study was patterned to a great extent after the one used by Jaffe et al (5) in the treatment of osteogenic sarcoma.

Blood samples were drawn at 24, 48, and 72 hours after the beginning of the MTX infusion to determine the plasma MTX concentration and serum creatinine levels. Creatinine clearances were determined for each 24-hour period following the MTX infusion during the CF rescue. Patients with evidence of delayed plasma MTX clearance (> 2×10^{-8} M at 72 hours) had the CF rescue prolonged until the plasma MTX was < 10^{-8} M.

The patient and disease characteristics are shown in table 1.

RESULTS AND DISCUSSION

The median time in the study was 5.9 weeks. The numbers of patients receiving the sequentially increasing MTX doses were as follows: 1500 mg/m² (28 patients), 3000 mg/m² (27), 4500 mg/m² (17), 6000 mg/m² (11), and 7500 mg/m² (nine).

One of the 28 patients (4%) achieved a partial response (defined as a reduction by at least 50% of the product of the longest perpendicular diameters of the most clearly measurable tumor lesion). The patient was a black male, 55 years of age, with an adenocarcinoma involving scalene nodes and a bronchial tumor. He had evidence of a partial remission in the scalene node on Day 12, was diagnosed as having progressive disease on Day 79, and died on Day 116 from the time he received his first dose of MTX. Four patients (14%) had no change in their disease while 23 patients (82%) had objective progression of disease (defined by > 50% increase in any measurable lesion or the appearance of new areas of malignant disease). Seventeen patients (61%) had metastases during treatment. These metastases are reported by site in table 2. Assuming that the patients are a representative sample, we are 98% confident that the true response rate for this therapy is < 20%. The estimate of median time to treatment failure was 4.7 weeks. Twenty-three

TABLE 1.—Patient and disease characteristics

Characteristic	No. of patients (%)
Age (yrs)	
< 50	5 (18)
50–59	12 (43)
60–69	9 (32)
> 70	2 (7)
Race	
White	22 (79)
Black	6 (21)
Sex	
Male	20 (71)
Female	8 (29)
Histology	
Squamous cell carcinoma	5 (18)
Adenocarcinoma	18 (64)
Large cell carcinoma	5 (18)
Extent of disease	
Limited	4 (14)
Extensive	24 (86)
Sites of disease*	
Lung	19 (68)
Lymph nodes	8 (29)
Liver	2 (7)
Kidney	2 (7)
Soft tissue	2 (7)
Breast	1 (4)
Initial ECOG PS	
0 (fully active)	9 (32)
1 (ambulatory)	14 (50)
2 (in bed < 50%)	5 (18)
Weight loss	
None	17 (61)
Weight loss	11 (39)
Prior radiotherapy	
No	16 (57)
Yes	12 (43)
Prior surgery	
No	15 (54)
Yes	13 (46)

*Patient may have > 1 site.

TABLE 2.—Additional metastases developed during treatment (28 patients)

Site	Frequency
Bone	5 (18%)
Soft tissue	4 (14%)
CNS	3 (11%)
Regional lymph nodes	2 (7%)
Skin	2 (7%)
Liver	1 (4%)

TABLE 3.—Distribution of patients by type and degree of treatment-related toxic effect

Toxic effect	No. of patients with toxic effects				
	None	Mild	Moderate	Severe	Life-threatening
Vomiting	14	9	5		
Diarrhea	22	3	3		
Other gastrointestinal	24	1	3		
Bleeding	27	1			
Skin and mucous membrane	24	2	2		
Respiratory	26	1		1	
Genitourinary	17	8	2	1	
Blood	19	4	4		1
Cardiac	27	1			

of 28 patients have died. The Kaplan-Meier (6) estimate of median survival was 17 weeks. The only statistically significant factor of the various patient and disease characteristics affecting survival was weight loss. The estimated median survival for patients with no weight loss versus those with weight loss was 20 and 10 weeks, respectively ($P = 0.055$, log rank test) (7).

Treatment complications were reported according to the ECOG standard by degree (none, mild, moderate, severe, or life-threatening), type, and cause. The distribution of patients by worst degree of treatment-related complications were as follows: none (five patients), mild (11), moderate (nine), severe (two), and life-threatening (one). The distribution of patients by type and degree of complication is given in table 3. The median values of wbc counts, platelet counts, and serum creatinine by course are shown in table 4. One patient experienced life-threatening bone marrow suppression (wbc count of 308/mm^3 and platelet count of 25,000/mm^3). Despite adequate hydration and alkalinization of the urine, the plasma MTX 72 hours from the initiation of the infusion of MTX was elevated (9.09×10^{-7} M). Of the two patients who experienced severe complications, one experienced acute renal failure 2 days after the third dose of MTX and the other experienced acute shortness of breath 10 minutes after the sixth infusion of MTX. The former patient, whose urine pH was maintained at > 7.0, had plasma MTX levels of 8.0×10^{-6} M and 2.21×10^{-7} M at 48 and 120 hours from the initiation of the MTX infusion, respectively. With prolongation of the CF rescue, the patient's renal function returned to normal. There were no deaths attributed to drug toxicity.

Djerassi et al (1) reported objective responses in nine of ten patients with non-small cell carcinoma of the lung after HDMTX-CF therapy. The responses occurred in two patients with adenocarcinoma and in seven patients with squamous cell carcinoma. In a later study, Djerassi et al (2) reported that in a group of 87 patients with lung cancer (34 with squamous cell carcinoma, 24 with adenocarcinoma, 11 with oat cell carcinoma, and 18 with miscellaneous other types) treated with HDMTX-CF therapy, 52 patients (82%) showed some degree of tumor reduction. Isacoff et al (3) reported on 18 patients with non-small cell carcinoma of the lung receiving HDMTX therapy: two of nine patients with adenocarcinoma, two of five with squamous cell carcinoma, and two of four with large cell carcinoma had objective remissions. The results of our study differ significantly from those mentioned above. These differences could be explained by the different administration schedules of HDMTX-CF therapy utilized. However, a more likely explanation of why our results differ from those of Djerassi et al is the difference in the definition of an objective antitumor response. Our results are in agreement with those reported by Minna et al (4) who found no objective responses in nine patients (six with adenocarcinoma and three with large cell carcinoma) receiving HDMTX-CF rescue.

The results of this study demonstrate that HDMTX-CF rescue is not effective in the treatment of patients with non-small cell carcinoma of the lung.

TABLE 4.—Median wbc counts, platelet counts, and serum creatinine, by course

Course	Median wbc values (cells/mm³)	No. of patients with wbc counts of < 4500 cells/mm³	No. of wbc count observations	Median platelet values (× 1000 cells/mm³)	No. of patients with platelet counts of < 200,000	No. of platelet observations	Median creatinine values (mg/100 ml)	No. of patients with creatinine > 1.2 mg/100 ml	No. of creatinine observations
1	8400	4 (18%)	22	326	3 (14%)	22	1.0	2 (9%)	22
2	7500	3 (14%)	22	340	1 (5%)	20	0.9	2 (10%)	21
3	7000	0	12	335	1 (8%)	12	0.9	2 (14%)	14
4	6700	0	8	315	0	8	0.9	2 (20%)	10
5	6600	0	7	350	0	5	0.9	0	7
6	7300	1	6	320	1	6	0.9	0	5
7	9400	0	2	241	1	2	1.0	0	2
8	7100	0	1	113	1	1	1.0	0	1
9	6500	0	1	243	0	1	1.4	1	1

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Fatal Hepatic Necrosis After High-Dose Chemotherapy Following Haloalkane Anesthesia[1]

Robert J. Spiegel,[2,*] Philip A. Pizzo,[2] Joseph C. Fantone,[2] and Hyman J. Zimmerman[3]

SUMMARY

We describe two patients who died with rapidly progressive hepatic necrosis following intensive chemotherapy and haloalkane anesthesia. Intensive multiple-drug chemotherapy has been associated with severe systemic toxic effects, but fulminant hepatic necrosis has not been recognized as a frequent complication. Furthermore, overt hepatic injury following the currently used halogenated anesthetic agents alone is rare without prior exposure and generally is considered to be idiosyncratic, ie, dependent upon unusual patient vulnerability. The unanticipated hepatic failure in these patients is reported to alert clinicians to this potential complication of intensive cancer chemotherapy and the possible additive adverse effect of haloalkane anesthesia.

[Cancer Treat Rep 64:1023–1029, 1980]

Although transient abnormalities of liver function are commonly associated with a variety of chemotherapeutic agents, fulminant hepatic failure as a consequence of chemotherapy is rare (1). We report an increased incidence of apparent hepatic injury during two recent trials of intensive combination chemotherapy which used drugs not generally considered to be hepatotoxins. Furthermore, two of the 17 patients treated had fatal hepatic necrosis. It is notable that most of the patients had recently received general anesthesia for biopsy, removal of bulk tumor, or autologous bone marrow storage. This report details the clinical course of the two patients who developed unanticipated fatal hepatic failure following intensive chemotherapy after exposure to haloalkane anesthetic agents. Although the drugs administered to these patients are not considered hepatotoxic at standard doses, the possibility that severe hepatic injury resulted when these drugs were combined at increased doses or when they potentiated the toxic effects of prior anesthesia warrants consideration.

CASE REPORTS

Patient 1

Patient 1 was a 22-year-old man with osteosarcoma who received 18 courses of high-dose methotrexate (250 mg/kg) over a 9-month period. He then developed a solitary pulmonary metastasis which was completely excised under nitrous oxide and enflurane anesthesia. This was his first exposure to haloalkane anesthesia. Liver scan, SGOT, SGPT, alkaline phosphatase, bilirubin, and clotting parameters were normal following surgery. Eleven days after surgery an intensive chemotherapy regimen was initiated, as shown in table 1. Seven days after completing this intensive drug schedule the patient became hypotensive. Total bilirubin rose from 0.5 to 8.6 mg/dl and SGOT increased from 11 to 4356 units/liter over a 3-day period. The patient became febrile and was treated with broad-spectrum antibiotics (cephalothin, gentamicin, and carbenicillin), although no specific infection had been demon-

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strated. HBsAg was negative. Three days later (Day 10 after completing chemotherapy), he developed intractable hypotension and died. Post-mortem examination revealed a diffusely bile-stained 2200-g liver. Histopathology demonstrated extensive centrilobular hepatocellular necrosis, with multiple peripheral and midzonal necrotic foci (fig 1A). Some sinusoids contained material suggestive of fibrin strands. Only minimal fatty change was evident, and there was no biliary obstruction, inflammation, or infection. No evidence of residual osteosarcoma was found.

Patient 2

Patient 2 was a 3-year-old boy who had stage IV disseminated neuroblastoma. He received two courses of combination chemotherapy as shown in table 1, with a subsequent reduction of his retroperitoneal tumor and return of urinary vanillylmandelic acid and catecholamines to normal. A post-treatment liver scan was normal. In preparation for more intensive chemotherapy, he had removal of bone marrow, under halothane anesthesia, for cryopreservation and storage. He had no prior history of haloalkane anesthesia. The next day he began an intensive chemotherapy regimen which included concurrent vincristine (2.0 mg/m^2) on Day 1, 4 days of cyclophosphamide (50 mg/kg/day), and 5 days of DTIC (250 mg/m^2/day). Three days after completing this course of therapy he developed biochemical evidence of hepatic injury: his SGOT rose from 63 to 1872 units/liter over 6 days and his bilirubin rose from 1.1 to 5.3 mg/dl during the same period. He became jaundiced, febrile, and developed anasarca. Treatment with broad-spectrum antibiotics was initiated although an infectious etiology was not found. HBsAg was negative. Eleven days after completing the intensive treatment schedule the patient had a respiratory arrest and could not be resuscitated. Post-mortem examination revealed a mottled liver which showed diffuse parenchymal destruction with severe central and midzonal coagulative degeneration and necrosis accompanied by marked sinusoidal dilation and congestion (fig 1B). No fibrosis, bile duct proliferation, obstruction, infection, or inflammation was present. Many residual hepatocytes showed microvesicular steatosis. No tumor involvement of the liver was found.

Other Cases (table 1)

Eight additional patients with metastatic osteosarcoma received therapy identical to that of patient 1. Six had surgery under halothane or enflurane anesthesia prior to chemotherapy, and two of these six showed biochemical evidence of hepatic injury following therapy. The other four patients receiving halothane or enflurane anesthesia prior to therapy had no significant liver abnormalities. The two patients without recent haloalkane anesthetic exposure tolerated therapy well with no hepatic dysfunction.

Seven patients with stage IV neuroblastoma received therapy identical to that of patient 2. The cause of death in all patients was progressive tumor or sepsis. One patient who received halothane anesthesia and had additionally received abdominal irradiation (1000 rad) was found to have veno-occlusive liver disease with areas of centrilobular hemorrhage at post-mortem examination. Two other patients received halothane or enflurane anesthesia just after completing chemotherapy. Both patients had acute transaminase elevations < 4 days later, but these resolved within 1 week. Unfortunately, their liver histopathology at autopsy was obscured by the presence of hepatic tumor and candidiasis in one patient, and profound pre-morbid shock with severe liver congestion in the other. Four other patients received identical chemotherapy without anesthetic exposure and had no hepatic abnormalities.

COMMENTS

Hepatic failure has been associated with intensive chemotherapy only infrequently; however, a number of agents are known to produce transient hepatotoxicity by a variety of mechanisms (1,2). For example, the administration of methotrexate in large single doses frequently results in acutely elevated transaminases, and prolonged administration can lead to fibrosis, steatosis, and cirrhosis (3–5). The dose-related clinical toxicity of methotrexate in man suggests that it is an intrinsic hepatotoxin (1), and in animal experiments, regular inhibition of regenerative ability has also been demonstrated (6). 6-Mercaptopurine alone (7) or with doxorubicin (Adriamycin) (8) has also been associated with a mixed hepatocellular injury, characterized by jaundice, steatosis, and necrosis (9). More recently, cytosine arabinoside administered with either anthracyclines or with 6-thioguanine has been associated with hyperbilirubinemia (10), hepatocellular degeneration (11), and veno-occlusive disease with a Budd-Chiari syndrome (12). DTIC, vincristine, and cyclophosphamide, the drugs shared by patients 1 and 2, as well as the mechlorethamine used in patient 1, appear to have little hepatotoxic potential (1). Furthermore, even the antineoplastic agents known to be capable of producing hepatic

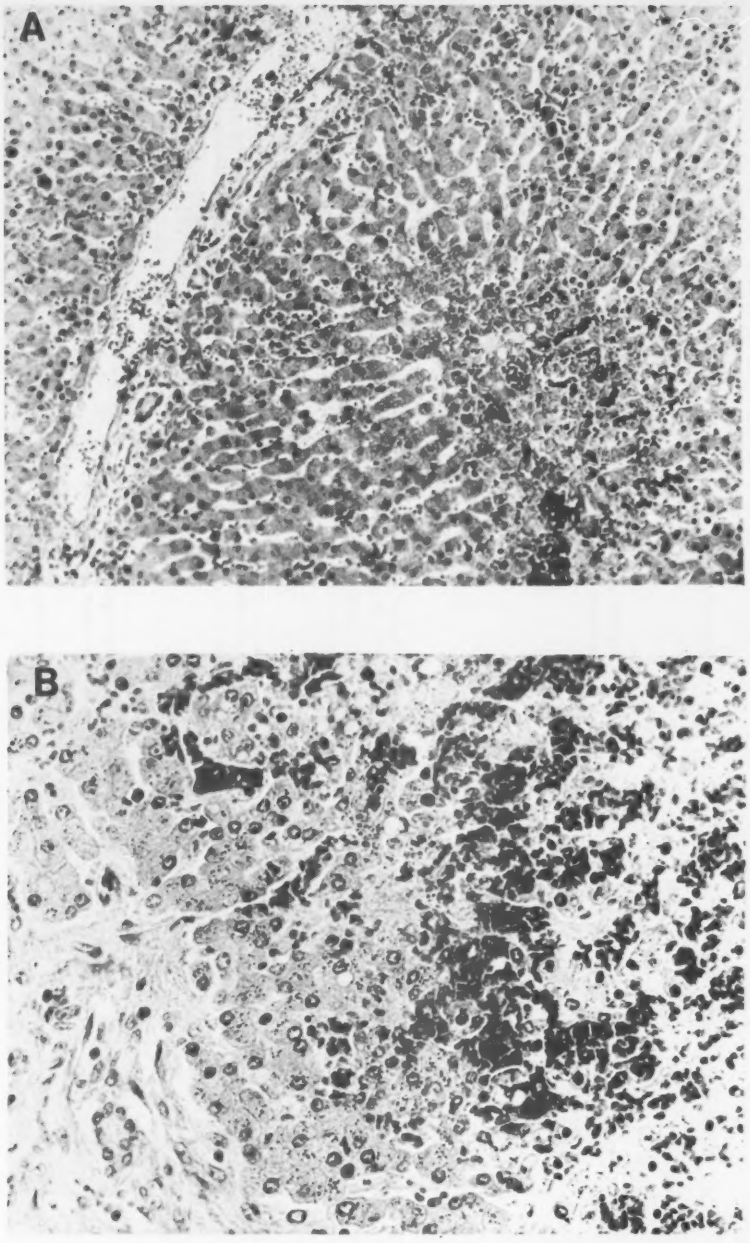

FIGURE 1.—A. Extensive hepatocellular necrosis, predominantly centrilobular with multiple periportal and midzonal foci. No inflammatory response and only minimal fatty change (hematoxylin and eosin stain, original magnification x 100). B. Diffuse parenchymal destruction. Severe centrilobular necrosis and congestion. No fibrosis or inflammatory reaction. Mild fatty metamorphosis (hematoxylin and eosin stain, original magnification x 250).

TABLE 1.—Patient characteristics and clinical course

Patient No.	Age	Sex	Anesthesia before or after chemotherapy	Index chemotherapy regimen*	Prior chemotherapy	Hepatic abnormalities following chemotherapy	Results
				Patients with hepatic necrosis			
1	22	M	Enflurane, 11 days before	1	High-dose methotrexate × 18	Day 28: rapidly deteriorating hepatic function with bilirubin to 8.6 mg/dl; SGOT to 4356 units/liter	Death on Day 30 with massive hepatic necrosis
2	3	M	Halothane, 1 day before	2	Vincristine, cyclophosphamide, and DTIC × 2	Day 10: rapidly deteriorating hepatic function with bilirubin to 5.3 mg/dl; SGOT to 1872 units/liter	Death on Day 16 with massive hepatic necrosis
				Patients with chemical hepatic dysfunction			
3	24	F	Enflurane, 34 days before	1	High-dose methotrexate × 7	Days 15–30: increased bilirubin to 4.0 mg/dl; alkaline phosphatase to 286 units/100 ml	Transient increase in SGOT/SGPT with subsequent methotrexate
4	14	M	Enflurane, 14 days before	1	None	Days 38–53: increased SGOT to 214 units/liter; SGPT to 357 units/liter	Transient increase in SGOT/SGPT with subsequent methotrexate
5	2	M	Enflurane, 1 day before	2 + abdominal radiation therapy	None	Days 2–17: increased bilirubin to 1.7 mg/dl; alkaline phosphatase to 284 units/100 ml	Death due to tumor progression, veno-occlusive changes at autopsy noted
6	15	M	Enflurane, 95 days after	2	Vincristine, cyclophosphamide, and doxorubicin × 2	Days 4–7 after enflurane: SGOT to 260 units/liter; SGPT to 520 units/liter; no abnormalities after initial chemotherapy	Death due to sepsis with *Pneumocystis carinii* and cytomegalovirus; at autopsy, liver showed only congestive changes
7	10	F	Halothane, 24 days after	2	Vincristine, cyclophosphamide, and doxorubicin × 2	No abnormalities after initial chemotherapy; SGOT to 231 units/liter; SGPT to 114 units/liter 1 day after subsequent halothane; then normal	Death due to sepsis with *Aspergillus* and *Candida*; at autopsy, liver involved with tumor and fungus
				Patients without hepatic dysfunction			
8	20	M	Enflurane, 41 days before	1	High-dose methotrexate × 8	None	No hepatic abnormalities
9	12	M	Halothane, 14 days before	1	High-dose methotrexate × 7	None	No hepatic abnormalities
10	35	M	Enflurane, 12 days before	1	High-dose methotrexate × 18	Days 5–10: increased SGOT to 77 units/liter; SGPT to 185 units/liter; then normal	No hepatic abnormalities
11	14	M	Enflurane, 28 days before	1	High-dose methotrexate × 1	None	No hepatic abnormalities

12	20	F	Fentanyl, 26 days before	1	High-dose methotrexate × 2, doxorubicin, cyclophosphamide, + 5-FU	None	Transient increase in SGOT/SGPT with subsequent methotrexate
13	15	M	Fentanyl, 26 days before	1	None	Days 9–10: increased SGOT to 64 units/liter; SGPT to 207 units/liter; then normal	No hepatic abnormalities
14	27	M	None	2 + abdominal radiation therapy	None	None	Death due to shock and *Candida sepsis*; no autopsy
15	11	M	None	2 + abdominal radiation therapy	None	None	Death due to sepsis with *Escherichia coli*; subcapsular hepatic bleed terminally; at autopsy, only hemorrhagic changes
16	1	M	None	2	None	None	No hepatic abnormalities
17	4	M	None	2	Vincristine and cyclophosphamide	Day 22: preterminal increased bilirubin to 8.4 mg/dl; alkaline phosphatase to 462 units/100 ml with hepatic involvement by tumor and fungus	Death due to sepsis with disseminated *Candida* which involved the liver

*Regimen 1: NIH protocol 77-3 for metastatic osteosarcoma. Day 1— methotrexate (7.5 g/m^2) as a 6-hr infusion followed by citrovorum rescue; Day 7—begin concurrent cyclophosphamide (40 mg/kg/day) for 2 days, doxorubicin (35 mg/m^2/day) for 5 days; Day 14—cyclophosphamide (40 mg/kg) and melphalan (0.4 mg/kg/day) for 2 days. Regimen 2: NIH protocol 75-8 for stage IV neuroblastoma. Vincristine (2.0 mg/m^2), DTIC (250 mg/m^2/day) for 5 days, and cyclophosphamide (50 mg/kg/day) for Days 2–4.

injury have only rarely been associated with frank hepatic failure.

The possibility that prior anesthesia resulted in hepatic injury merits consideration. Halothane is known to produce severe hepatic injury in a small proportion of exposed individuals. However, clinical toxicity is quite rare, with only slightly more than 900 cases reported in the English language literature by 1976 (1). The incidence of hepatic injury has been estimated to be approximately one in 2500 exposures (13). Nevertheless, halothane must always be regarded as having hepatotoxic potential. Halothane can regularly produce hepatic injury in several species of animals (1), and studies in man have shown mild hepatic impairment with elevated transaminase levels and decreased sulfobromophthalein excretion occurring in normal volunteers and persisting for at least 7 days after a first exposure to halothane (14). Enflurane, a chemically related anesthetic, has also been incriminated in several instances of post-operative hepatic injury, although its role in producing liver damage is far less clear than that of halothane. The causal relationship of these agents to clinical instances of post-operative liver damage is frequently difficult to ascertain, however, and remains controversial (15). Nevertheless, it is generally agreed that halothane toxicity is more prevalent in adults, < 10% of reported cases occur in patients < 20 years old (16). Furthermore, it is associated with a history of prior exposure in at least 75% of cases. The low incidence and the associated clinical features indicate that despite their toxic potential the hepatic injury produced by these agents is usually idiosyncratic rather than truly hepatotoxic.

The cause of hepatic necrosis in the two cases presented remains unknown. They have in common a recent course of anesthesia and prolonged intensive exposure to chemotherapeutic agents that would not be expected to produce this form of hepatic injury under normal circumstances. Both cases demonstrated similiar changes in the liver at autopsy, consistent with toxic hepatocellular necrosis. However, the role of specific toxic agents, prior therapy, the possible sensitizing role of anesthesia, drug potentiation, or cumulative dose toxicity must remain speculative. Drug-related hepatotoxicity is notoriously difficult to substantiate reliably, even in a relatively simple clinical setting in which a single drug is suspected. With multiple drugs, particularly antineoplastic agents which are frequently employed in combination and in patients with multiple medical problems, this difficulty is compounded. However, in the two cases presented, the histopathologic findings of characteristic central zonal necrosis and the acute clinical presentation with fever and transaminase elevations > 1000 units/liter, occurring 8 and 18 days after anesthesia, are suggestive of an acute toxic injury that could be the result of the haloalkanes, the specific chemotherapeutic agents, or their combination.

The rarity of hepatic injuries attributable to halogenated anesthetics, particularly after a first exposure, as well as the improbability of hepatotoxicity due solely to the antineoplastic agents, suggests that the unusual circumstances of these cases warrant further consideration. It is possible that prolonged intensive chemotherapy may render patients susceptible to severe hepatotoxicity from compounds normally innocuous or only mildly hepatotoxic, or may increase the incidence of hepatic injury with idiosyncratic hepatotoxins. Conversely, anesthetic agents may produce low grade or subclinical hepatic injury, altering drug metabolism and rendering the liver more susceptible to subsequent drug-induced hepatic cellular injury. The cases described in this report indicate the need to be alert to potential hepatotoxicity that may follow high-dose polychemotherapy, particularly in the post-anesthetic setting. Thorough documentation of hepatic dysfunction in such cases may serve to elucidate the mechanism of hepatotoxicity and the exact factors involved.

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Preclinical Toxicologic Study of 2,3-Dihydro-1*H*-imidazo[1,2-*b*] pyrazole (IMPY) in Mice, Dogs, and Monkeys[1,2]

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SUMMARY

Single-treatment schedules in mice and dogs and multiple-treatment schedules in dogs and monkeys were used to evaluate the toxicity of 2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole. The LD50 of the iv single dose in male and female mice collectively was 993 mg/kg (2980 mg/m²). The major target organs in mice, dogs, and monkeys were the bone marrow, lymphoid tissue, and gastrointestinal tract. Clinical signs at lethal and high toxic doses were weight loss, diarrhea, hematochezia, emesis, anorexia, mydriasis, dyspnea, lethargy, and stupor. The immediate toxic effect on blood cells was a depression of rbcs with suppression of lymphoid elements occurring later. In dogs, the most toxic schedule was single bolus injections. Attenuation of toxic responses occurred if rest periods were introduced between single or repeated daily dose schedules. The monkeys were more sensitive than the dogs to the high toxic dose on a milligram per meter squared basis, with similar sensitivity to the low toxic dose in the repeated daily injections.

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2,3-Dihydro-1*H*-imidazo[1,2-*b*]pyrazole (IMPY) is a completely new ring type for antitumor agents. The molecule contains an imidazoline and a pyrazole ring and there is no chemical relationship to either mercaptopurine or to representatives of the tumor-inhibiting pyrazolo-pyrimidines. IMPY selectively inhibits DNA synthesis, but does not inhibit RNA or protein synthesis in S180 ascites cells in vivo and in cultured L (1) and HeLa cells (2). The inhibition of DNA synthesis is rapidly reversible after 16–24 hours of exposure, is prevented and reversed by deoxyguanosine, and is potentiated by deoxycytidine (1). This paper presents a preclinical toxicologic evaluation of this new antitumor agent in mice, dogs, and monkeys.

MATERIALS AND METHODS

Experiments were performed on BDF_1 mice, beagle dogs, and rhesus monkeys in accordance with the procedures outlined by Prieur et al (3) and modified by the Battelle Columbus Laboratories, Toxicology Program Office.[6] Dogs were supplied by the Mammalian Genetics Center, National Institutes of Health, Bethesda, MD, through the Battelle Toxicology Program Office, and monkeys were purchased from a commercial supplier. Dogs and monkeys were housed in individual steel cages and provided with water ad libitum. Prior to the study, fecal examinations for parasites were performed for dogs and monkeys. The monkeys were held in isolation for a minimum of 6 weeks and were tuberculin-negative in three consecutive tests before they

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[6]Procedures Manual. Battelle Columbus Laboratories, OH, Feb 5, 1975.

TABLE 1.—Treatment schedules and daily dose ranges used for toxicologic evaluation of IMPY in mice, dogs, and monkeys

Species	Treatment schedule	Daily dose range (mg/m^2)
Mouse (BDF_1)	Single injection	2385–4800
Dog	Single injection	250–8000
	Daily for 5 days	250–4000
	Daily on Days 1–5, 15–19, 29–33 with rest periods on Days 6–14, 20–28	250–2000
	Once per wk for 6 wks	500–4000
	Daily for 10 days	125–2000
Monkey	Daily for 5 days	249–1992

were released for treatment. Dogs and monkeys had a preclinical toxicologic evaluation that included physical and ophthalmic examinations, hematologic and serum chemistry determinations, urinalyses, necropsies, and histopathologic evaluations. At least two complete hematologic and serum chemistry determinations were performed on each animal before treatment. Both dogs and monkeys were treated in pairs, one male and one female at each dose level. Animals were observed for 45 days after treatment. The treatment schedules used in this study are shown in table 1.

Male and female BDF_1 mice were supplied by the Mammalian Genetics Center, National Institutes of Health. They were housed in polycarbonate cages containing hardwood bedding. Commercial mouse chow and water were available ad libitum. Single iv doses of IMPY were administered to 13 animals of each sex; each animal weighed between 20 and 21 g. Only ten males and ten females per dose level were used in the calculation of the median lethal dose (LD50); three animals per sex served as satellite groups. If an animal died unobserved during the 14-day observation period, an animal from the satellite group was necropsied at that time for histopathologic evaluation.

The chemical identity of IMPY[7] was verified by infrared spectroscopy and melting point determination. The drug was stored at 4°C. The solubility in nonpyrogenic sterile distilled water was > 500 mg/ml. IMPY was not formulated with sodium chloride since such solutions are prone to discoloration after storage at elevated temperatures. Solutions of 500 and 100 mg/ml were used for in vivo blood compatibility studies. A concentration of 100 mg/ml was prepared for the protocol studies immediately before iv treatment.

[7]IMPY was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

The following definitions of dose levels were used in this study:

Highest nontoxic dose (HNTD)—the highest dose at which no hematologic, chemical, clinical, or morphologic drug-induced alterations occur; doubling this dose produces the aforementioned alterations.

Toxic dose low (TDL)—the lowest dose which produces drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose produces no lethality.

Toxic dose high (TDH)—the dose which produces drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose produces lethality.

Lethal dose (LD)—the lowest dose which produces drug-induced death in any animal during the treatment or observation period.

RESULTS

A drug concentration of 500 mg/ml was compatible with canine serum or plasma. This concentration, however, produced a brown coagulate in whole blood, even at 1:16 dilutions of this drug solution in blood. A 100 mg/ml solution produced hemolysis in whole blood down to 1:4 dilutions. The defined toxicity levels in mice, dogs, and monkeys are given in table 2.

Median Lethal Dose in Mice

The 14-day LD50s for male and female BDF_1 mice after single iv injections of IMPY were 978 and 1026 mg/kg (2934 and 3078 mg/m^2), respectively. The collective LD50 for both sexes was 993 mg/kg (2980 mg/m^2). Survival time for female mice was not markedly affected by dose level but an inverse relationship between survival time and dose level may have been apparent for male mice. Most of the an-

TABLE 2.—Defined* toxicity levels of IMPY in mice, dogs, and monkeys

Species (males and females collectively)	Study	Daily dose mg/kg	Daily dose mg/m^2
Mouse	Acute toxicity		
	LD90	1107.0	3321
	LD50	993.0	2980
	LD10	891.0	2674
Dog	Single dose		
	LD	400.0	8000
	TDH	200.0	4000
	TDL	25.0	500
	HNTD	12.5	250
	5 daily doses		
	LD	200.0	4000
	TDH	100.0	2000
	TDL	25.0	500
	HNTD	12.5	250
Monkey	5 daily doses		
	LD	166.0	1992
	TDH	83.0	996
	TDL	41.5	498
	HNTD	20.75	249

*See ref 3.

imals died approximately 2–4 days after treatment; males given the lower doses tested survived a few days longer. Toxic signs at lethal doses included hyperactivity immediately after injection, prostration within 30 seconds, loss of righting reflex, ataxia, hyperpnea, ptosis, ruffled fur, a slow response to stimuli, and reddish brown urine. Maximum loss of body weight occurred 2–6 days after treatment. Mice necropsied 14 days after treatment gave no evidence of drug-related lesions. Animals dying within 2–6 days showed the following histopathologic changes: necrosis and vacuolation of hepatocytes, interstitial hemorrhage and cellular vacuolation in the proximal tubules of the kidneys, edema and hemorrhage in the lung parenchyma, and cellular necrosis and atrophy in the spleen, lymph nodes, and thymus. Bone marrow sections were hypocellular with elevated myeloid:erythroid ratios.

Single-Dose Study in Dogs

Six pairs of dogs were injected iv with single IMPY doses of 400, 200, 100, 50, 25, or 12.5 mg/kg (8000, 4000, 2000, 1000, 500, or 250 mg/m^2). The two dogs treated with 400 mg/kg of IMPY were found dead with bloody exudate from the mouth within 24 hours. Hematologic and blood chemistry determinations were not done prior to death; urine aspirated from the bladder after death contained blood. Mydriasis, ataxia, anorexia, and emesis were the clinical signs of toxicity at a dose of 200 mg/kg. Anorexia or reduced food consumption and emesis in one dog were observed at the 100-mg/kg dose level. Hematochezia was observed in one dog injected with 25 mg/kg of IMPY. Clinical signs of toxicity were not seen at the 12.5-mg/kg dose.

Changes in blood chemistry values were not remarkable after single iv doses. Decreases in hematocrit, hemoglobin, rbc count, and wbc count values (with a later compensatory leukocytosis), and hematuria were observed at a dose of 200 mg/kg. Lower doses produced depressions of circulating blood cells that were not dose-related. At the lowest dose administered (12.5 mg/kg), blood cell measurements were slightly depressed, compared to baseline values, on Day 2 (rbcs) and Days 4 and 8 (wbcs). Because of the lack of dose-response relationships, these mild changes were not considered to be drug-related.

Lymphoid tissue necrosis, bone marrow depression, and hemorrhages in tissues occurred at the LD. Histopathologic lesions were less severe at lower doses and included swelling of hepatocytes, splenic follicular atrophy, and necrotic changes in lymph nodes. At the 25.0-mg/kg dose level, histopathologic findings in one dog that excreted bloody stools included hemorrhages in the brain, choroid plexus, and kidneys, and depletion of erythroid ele-

ments in the bone marrow. Histologic lesions were not apparent at the 12.5-mg/kg dose level.

Five Daily Dose Study in Dogs

Six pairs of dogs were used in this study. One pair was used as a control and five pairs were treated with IMPY once daily for 5 consecutive days at the following levels: 200, 100, 50, 25, or 12.5 mg/kg/day (4000, 2000, 1000, 500, or 250 mg/m²/day). Dogs treated with 200 mg/kg/day died after the third or fourth dose. Anorexia, adipsia, diarrhea, emesis, weight loss, weakness, lethargy, shallow respiration, chest rales, tremors, reddish brown urine, and mydriasis were observed at the two highest dose levels. Onset of toxic signs at the 100-mg/kg dose was delayed until Day 4.

Slight increases or decreases in rbc count values and leukocytosis were observed at the highest dose level. Marked increases in BUN, sulfobromophthalein retention, and alkaline phosphatase (AP) were apparent just prior to death. At the 100-mg/kg dose level, similar changes in serum chemistry values were present; creatinine, bilirubin, SGOT, and SGPT increased, and sodium, potassium, and chloride levels decreased. Marked decreases in the hematocrit, hemoglobin, rbc count, and platelet count values occurred, and leukocytosis was also present. Wbc depression was a delayed effect. Slight to moderate depressions of rbc and wbc levels occurred at the 50- and 25-mg/kg dose levels. At the 12.5-mg/kg dose level, one dog showed persistent weight loss with slightly increased AP levels from Day 24 to Day 52; the other dog showed no clinical or biochemical signs of toxicity. Weight loss and slightly increased AP values seen for the one dog receiving a dose of 12.5 mg/kg may or may not have been drug-related. Slight progressive weight loss was observed for this dog during the pretest period.

Brain, kidney, and lung hemorrhages, generalized lymphoid depletion, bone marrow depression, vacuolation of hepatocytes, and pigment deposition in hepatic canaliculi were observed in dogs treated with 200 mg/kg of IMPY. At the 100-mg/kg dose level, histologic lesions were observed only in the animal killed on Day 6; they included hemorrhages in the kidney and lymph nodes, lymphoid necrosis and depletion, bone marrow depression, and excess bile in the hepatic canaliculi. The dog that lost weight at the 12.5-mg/kg dose level had lymphoid depletion in the thymus and lymph nodes and swollen centrilobular hepatocytes. These lesions may or may not have been drug-induced. Histopathologic lesions were not seen for the other dog receiving the 12.5-mg/kg dose.

Five Daily Dose Study in Monkeys

Five pairs of monkeys were used for this study. One pair was used as a control and four pairs were treated with IMPY once daily for 5 consecutive days at the following dosage levels: 166, 83, 41.5, or 20.75 mg/kg/day (1992, 996, 498, or 249 mg/m²/day). The female died after the fourth dose of 166 mg/kg; the male died after receiving the full series. Weight loss, diarrhea, emesis, anorexia, dark brown urine, mydriasis, head twitching, dyspnea, stupor, and hypothermia occurred in monkeys after the second dose of 166 mg/kg and became more severe with subsequent doses. Similar but less prominent signs were noted at the 83-mg/kg level.

A moderate decrease in hematocrit, hemoglobin and rbc count values and marked increases in BUN values were observed at the two highest dose levels. Lymphopenia occurred in both monkeys at the 166-mg/kg dose level and in one monkey at the 83-mg/kg dose level. Slight to moderate increases in SGOT, SGPT, and bilirubin values occurred at these levels. Marginal decreases in rbc counts were observed at the 41.5-mg/kg dose level and leukocytosis or a delayed leukopenia was also present.

Histologic lesions were most prominent in monkeys treated at the two highest dose levels. Monkeys treated at the 166-mg/kg dose level had congestion, edema, and hemorrhages in the intestinal mucosa, spleen, brain, kidneys, and adrenal glands; generalized lymphoid depletion, depression, and pyknosis of the bone marrow; and hepatocellular vacuolation and necrosis. Tissue changes at the two lowest doses occurred only in animals killed 24 hours after the fifth dose. Lesions observed at the 83-mg/kg dose level were hemorrhagic lymph nodes and interstitial hemorrhages in the kidney, bone marrow depression, and severe fatty change in the liver. Tissue changes at the 41.5-mg/kg dose level were bone marrow depression and vacuolation of hepatocytes.

Multiple-Dose Study With Rest Periods in Dogs

Three pairs of dogs were used in this study. The treatment schedule was five consecutive daily injections of IMPY for three treatment periods with intervening 9-day rest periods. Dose levels used were the defined TDH, TDL, and HNTD from the five daily dose study: 100, 25, or 12.5 mg/kg/day (2000, 500, or 250 mg/m²/day). Toxic signs at the 100-mg/kg dose level were diarrhea, emesis, anorexia, weight loss, lethargy, muscular weakness, and dark brown urine. The dogs excreted dark urine and were lethargic during the rest period. During the second and third treatment periods, these signs

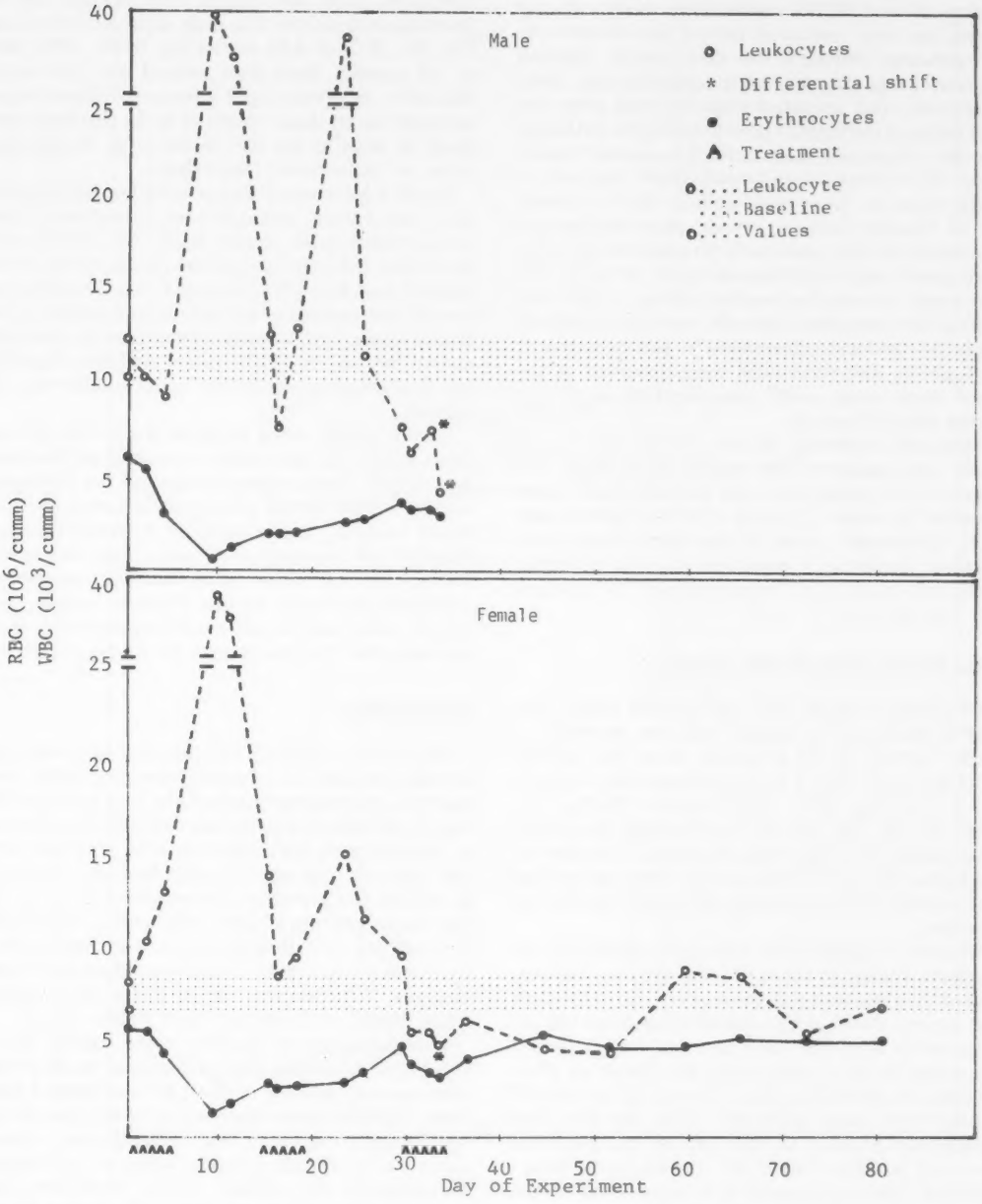

FIGURE 1.—Peripheral wbc and rbc counts after iv injection of 100 mg/kg of IMPY in dogs: 5 daily doses repeated 3 times.

were mild or absent. No manifestations of clinical signs were observed at the two lower doses.

Changes in serum chemistry values were confined to dogs treated at the highest dose level. Serum AP and SGOT values were increased only during the first treatment period; the elevation in AP persisted through the rest period. Marked depressions of the rbc count, neutrocytosis, reticulocytosis, and crenated rbcs occurred after the first series of the highest dose level (fig 1). Although the rbc values had not returned to control values when the second series began, there was only a slight effect on these blood cells in this treatment period. Leukocytosis was present after the first two treatment periods; leukopenia was present after the third period with a differential shift. Although the wbc count returned to baseline values, weight loss during the treatment periods was not regained. Slight to moderate decreases in the hematocrit, hemoglobin, and rbc values were observed at the lower dose levels, with reticulocytosis occurring during the rest period.

Lymphoid depletion in the thymus and lymph nodes, congestion of the colonic epithelium, and Kupffer cell hyperplasia and hemosiderosis were observed in animals treated with the highest dose level. Histologic lesions at the lower doses were lymphoid depletion, congestion, and hemosiderosis in the spleen and slight hyperplasia of the intralobular bile ductules.

Once Weekly Dose Study in Dogs

Two pairs of dogs were used in this study. The IMPY treatment schedule was six consecutive weekly injections. Dose levels were the defined TDH and TDL from the single-dose study in dogs: 200 or 25 mg/kg (4000 or 500 mg/m^2). Bloody diarrhea, emesis, and reduced food consumption were most severe after the first and second injections at the higher dose level. These signs were reduced at the lower dose level, and only one animal had bloody diarrhea.

Changes in hematologic and serum chemistry values were similar to those of other studies. Depression of the rbc count was most severe after the first and second doses at the higher dose level (fig 2). Wbc counts were increased after the first or second injections or both; leukopenia was observed after subsequent treatments. Elevations of AP and SGOT values were most prominent after the first two treatments. Similar, but less severe, changes were observed in dogs treated with the 25-mg/kg dose.

Tissue changes observed were hemorrhage in the spleen and lymph nodes, thymus atrophy, and hyperplasia of the Kupffer cells with hemosiderosis.

Ten Daily Dose Study in Dogs

Four pairs of dogs were used in this study. The IMPY treatment schedule was ten consecutive daily injections. Daily dose levels were one half the defined doses from the five daily dose study in dogs: 100, 50, 12.5, or 6.25 mg/kg/day (2000, 1000, 250, or 125 mg/m^2). Both dogs treated with 100 mg/kg died after receiving eight injections. Clinical signs were similar to those observed in the five daily dose study in dogs at the 200-mg/kg level. Toxic signs were not observed at lower doses.

Marked decreases in hematocrit, hemoglobin, and rbc count values, and increases in nucleated rbcs, wbcs, reticulocytes, serum BUN, AP, SGOT, total and direct bilirubin, and serum ions occurred at the highest dose level. Dogs treated with 50 mg/kg had severe depressions of rbc counts and moderate reticulocytosis, with a marginal decrease in wbc count and an increase in SGOT value. At lower dose levels, a slight decrease in rbc count values was observed.

Tissue lesions were most severe at the LD and in dogs killed 24 hours after treatment at the lower dose levels. Diffuse hemorrhages in the lungs and spleen, emboli in the pulmonary arteries and skin blood vessels, vacuolation of hepatocytes, and Kupffer cell hemosiderosis were observed at the 100-mg/kg dose level. Bone marrow depression, lymphoid depletion in the thymus, spleen, and lymph nodes, and Kupffer cell hemosiderosis were present after treatment with 50 mg/kg of IMPY.

DISCUSSION

The toxicity of IMPY was manifest in tissues possessing populations of rapidly dividing cells: bone marrow, gastrointestinal mucosa, and lymphoid tissue. Toxic effects on bone marrow cells were limited to granulocytes and erythroid cells; platelets were not affected. Damage to circulating rbcs was suggested by the presence of hemoglobin in urine, hemosiderosis in phagocytic cells, and in vitro hemolysis of dog rbcs. Hepatotoxicity was characterized by increases in serum chemistry values and tissue changes. Toxicosis was similar in the three species (mice, dogs, and monkeys) used in this study.

Manifestations of toxicity were similar in all treatment schedules used in dogs and monkeys and were usually present at the LDs and highest toxic dose. Clinical signs observed at these dose levels were weight loss, diarrhea, hematochezia, emesis, anorexia, mydriasis, dyspnea, lethargy, and stupor. Increases in AP, SGOT, BUN, creatinine, and serum ion levels were present at the higher dosage levels but were reduced or absent at doses that

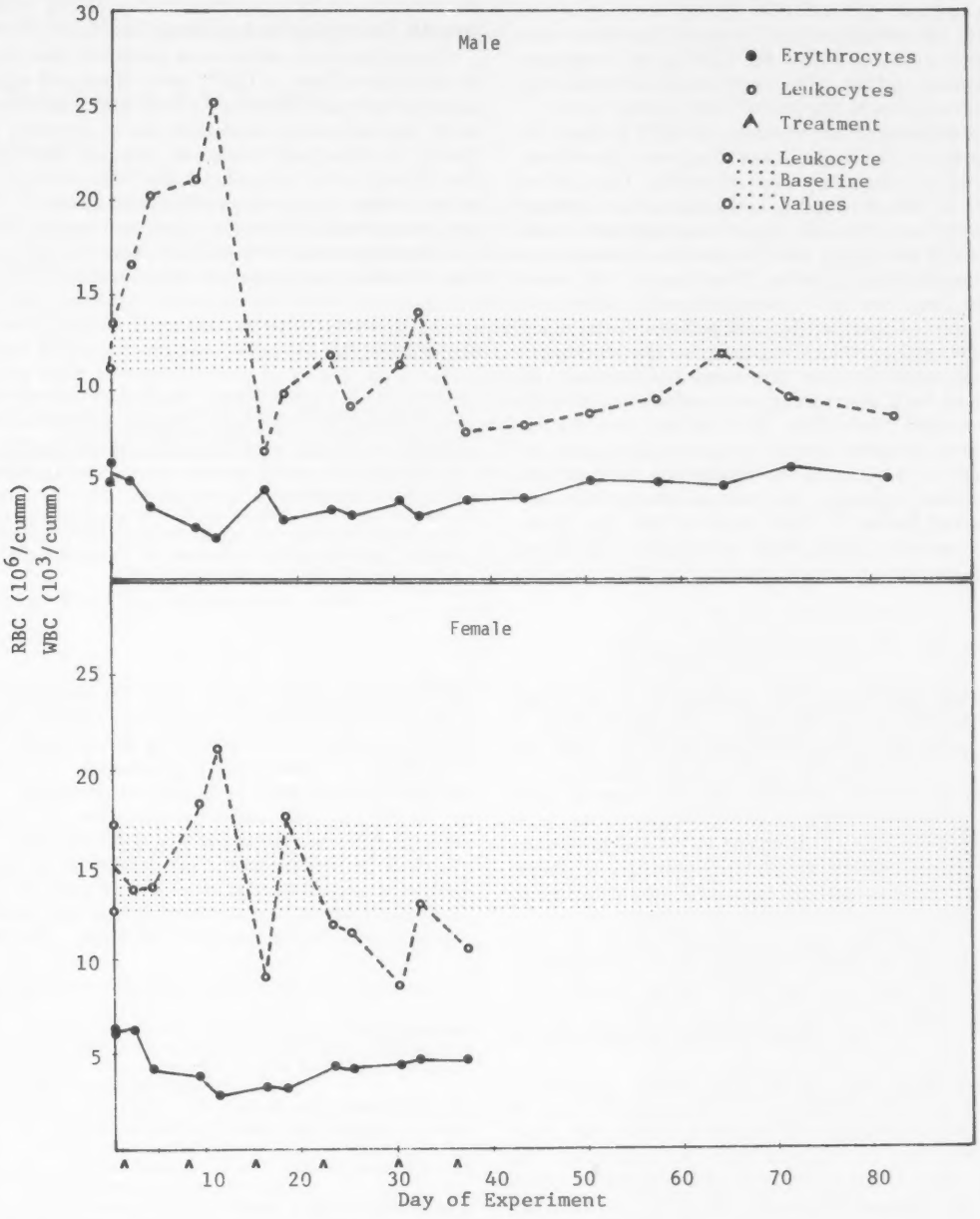

FIGURE 2.—Peripheral wbc and rbc counts after iv injection of 200 mg/kg of IMPY in dogs: 6 weekly doses.

produced no observable clinical signs. Changes in serum chemistry values occurred only on repeated dose schedules and were not observed after single treatments. Signs of gastrointestinal toxicity were also confined to the higher dosage levels (LD and TDH). Hematopoietic and lymphoid depletion were present at doses below the TDH in all treatment regimens, and the effect of erythroid elements was observed even at the lowest toxic dosage level.

Schedule-dependent toxicity of IMPY in dogs was apparent in the larger doses which could be administered on repeated dose schedules. Cumulative doses of 600–800 mg/kg were required to produce lethality on a five daily injection schedule whereas a dose of 400 mg/kg was lethal when administered as a single bolus injection. Much larger total doses of the drug can be tolerated if the five daily dose regimen is separated by rest periods. Total doses of 1500 mg/kg produced toxic signs but not lethality in dogs when given in three series of five daily injections with intervening rest periods. Six weekly treatments (total dose, 1200 mg/kg) also did not produce lethality, and the results suggest that the toxicity of the drug is not cumulative. In these two treatment regimens, the most severe toxicity was observed during the first week or first two treatment periods. After these treatments, toxic signs were reduced or absent, suggesting that dogs become tolerant to the toxic effects of IMPY. In contrast, repeated daily treatments with smaller doses without intervening rest periods do not reduce toxicity. Administration of the total amount of the LD from the five daily dose study in dogs as ten evenly divided doses produced lethality.

Rhesus monkeys were more sensitive than dogs to the toxic effects of IMPY when dose was calculated on body surface area. On a weight basis, however, approximately equivalent doses produced lethality on a five daily treatment schedule. Although the LD was lower in monkeys, the TDL and HNTD were similar on a body surface area basis. Toxic signs were similar in the two species. Lymphopenia was more pronounced in monkeys than in dogs, but the reticulocytosis response was less marked.

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Epidermal Cell Kinetics in Hairless Mice After Bleomycin. II. Perturbations After Multiple Doses[1,2]

E. Thorud* and O. P. F. Clausen[3,4]

SUMMARY

Bleomycin (BLM) at doses of 0.1 mg/animal was injected into hairless mice every 4 hours for 4 days. Epidermal cell cycle phase distributions were determined by flow cytofluorometric DNA measurements. Labeling indices and mean grain counts were determined by means of autoradiography, and mitotic rates were determined by demecolcine arrest of metaphases. A small, partially reversible accumulation of cells with G_2 phase DNA content was observed subsequent to BLM administration. Most of the cells accumulated in G_2 phase, however, seemed to stop proliferation and die in this phase. Thus, BLM does not fulfill the criteria for a good synchronizing agent in the hairless mouse epidermis. Flow cytofluorometry alone cannot determine whether piling up of cells in a particular cell cycle phase is true synchrony or an accumulation of lethally damaged cells. Several cell kinetic methods should be used to demonstrate real synchrony.

[Cancer Treat Rep 64:1039–1045, 1980]

Bleomycin (BLM) is an antineoplastic antibiotic isolated from *Streptomyces verticillus* (1). It has a well-documented growth inhibitory effect on some malignant tumors, and particularly on squamous cell carcinoma in man. The good clinical effect on squamous cell carcinoma and the side effects on mucosal stratified epithelia, skin and lung tissue, seem to be related to a relative lack of BLM-degrading enzymes in these tissues leading to a long-lasting and high concentration of active BLM (2,3). Clinically, BLM has been administered as a single agent (4), in combination with irradiation (5), and in combination with several other drugs (6). Different modes of administration have been used: eg, im or iv injections two to three times a week (7), iv or intra-arterial perfusions lasting for several days (8,9), or intracavitary infusions (10). Cell kinetic considerations have been used as a basis for various series of chemotherapeutic schedules. BLM has been considered by some investigators as a synchronizing agent with the ability to block cells reversibly in the G_2 phase (11–13) and has for this purpose been used prior to a phase-specific chemotherapeutic agent. BLM has also been used as a cell cycle phase-specific cell killer after presumed synchronization with vincristine (14).

Previous reports have shown that BLM has a complex effect on epidermal cell kinetics in hairless mice, and that it prolongs the maturation time of the differentiating cells (15–17). In the present study we are investigating the possible synchronizing effect of BLM when given continuously for one entire cell cycle.

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[4]We are grateful to Audhild Naavik, Aasa Schjølberg, Anne Cromarty, and Eva Moen for skillful technical assistance, and to Liv Støttum for typing the manuscript.

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TABLE 1.—Cell kinetic alterations in epidermis of

Time of measurement	LI			Cells with S-phase DNA content		
	BLM	Controls	% of controls	BLM	Controls	% of controls
Days of BLM administration						
I	5.4±0.5*	5.1±1.5	107.5±10.3	11.7±0.5	10.4±0.7	112.5±4.9
II	7.7±0.4	8.5±3.5	90.8±4.3	10.6±0.8	10.8±1.2	98.4±7.4
III	7.7±0.7	9.5±0.9	80.5±7.1	11.4±0.8	10.7±0.6	106.5±7.5
IV	8.1±0.5	9.2±0.5	88.0±5.2	12.1±0.5	14.2±0.4	84.7±3.5
Time after last BLM injection						
6 hrs	5.6±1.1	5.6±1.3	101.4±19.0	12.3±1.2	12.6±0.9	97.5±9.5
12 hrs	5.4±0.4	7.0±0.4	78.0±5.3	9.8±0.8	12.6±1.2	77.6±6.3
18 hrs	6.3±0.5	10.3±0.8	61.2±5.1	15.8±0.3	15.9±0.2	99.1±1.9
1 day	6.7±0.8	6.9±0.7	97.4±11.4	12.3±1.1	12.2±1.2	100.4±9.0
2 days	7.3±1.0	7.2±0.6	101.7±13.6	11.6±0.8	13.7±0.5	85.0±5.8
3 days	5.4±1.0	6.9±0.7	79.0±14.0	13.6±0.6	14.6±1.4	92.7±4.1
4 days	7.6±1.5	8.5±0.3	89.4±17.9	12.3±0.2	11.2±0.2	110.5±1.8
6 days	4.1±0.9	6.5±0.2	62.0±14.4	12.8±1.6	9.5±0.2	134.1±16.8
9 days	6.5±0.2	6.2±0.4	104.0±3.9	10.6±0.3	8.5±0.6	124.1±3.5

*Mean ± SEM.

MATERIALS AND METHODS

Animals.—Hairless mice of the hr/hr Oslo strain (18) (both sexes, 60–90 days old, and weighing about 25 g) were used in all experiments. Eight animals were kept in each cage and had free access to food (standard diet, Felleskjøpet A/S, Oslo, Norway) and water. The mean generation time of progenitor basal cells in hairless mouse epithelium is 50–55 hrs (18).

Bleomycin administration.—BLM (Lundbeck & Co, A/S, Copenhagen, Denmark) was dissolved in 0.9% NaCl and was injected ip with a dose of 0.1 mg in 0.5 ml of saline every 4 hours for 4 days. The injections were started at noon on Day 1. Control animals were injected with 0.5 ml of saline. Four animals in each cage served as controls and the other four were injected with BLM ip. Comparisons were made between animals from the same cage, and the results were expressed as percent of controls. All animals were killed by cervical dislocation at noon on the days indicated, with the exception of those killed on Day IV at 6, 12, and 18 hrs after the last BLM injection. The animals that were killed during the injection period, on Days I–IV, always received the last BLM injection 4 hrs before being killed.

Flow cytofluorometric DNA measurements.—Isolated basal cells were prepared according to the method of Laerum (20) and processed for flow cytofluorometric DNA measurements. This includes RNase treatment and staining with ethidium bromide as described in previous papers (21,22). The relative DNA content was determined with an ICP-11 flow cytophotometer (Phywe AG, Göttingen, W Germany), and the cell cycle distributions were calculated by means of a planimetric method (22).[5]

Labeling indices and grain count distributions.—Thirty minutes before being killed, groups of animals were injected with 30 microcuries of ^{3}H-thymidine (^{3}H-TdR) (specific activity, 6.7 curies/mmol, New England Nuclear Corp, Boston, MA) in 0.3 ml of saline. Basal cells were isolated as described above, and aliquots from single-cell suspensions were smeared on glass slides. After air-drying and fixation in absolute methanol for 20 minutes, the smears were dipped in a Kodak NTB 2 film emulsion diluted with distilled water (1:1), exposed for 3 weeks at 4°C, developed, and stained with hematoxylin-eosin. Labeled cells were counted per 1000 cells, and the lower limit for accepting a cell as labeled was based on the background activity and set at 3 grains over the nucleus. Grain counts were made from 150–200 labeled nuclei from three to four animals at certain times after the last BLM injection.

Mitotic rates.—Four hours before being killed, groups of animals were injected ip with 0.15 mg (1 mg/ml) of demecolcine (Ciba, Basel, Switzerland). The skin was immediately flayed off, fixed in Bouins solution for 4 hours, and dehydrated. Skin pieces were embedded in paraffin, cut in 5-μm-thick sections, and stained with hematoxylin-eosin. The mitotic rate was determined by counting accumulated metaphases among 1000 basal cells in the interfol-

[5]Gøhde W. Zellzyklusanalysen mit dem impulscytophotometer. Der einfluss chemischer und physikalisher noxen auf die proliferationskinetik von tumorzellen. Thesis. Münster, W Germany, 1973.

hairless mice during and after BLM administration

Cells with G_2-phase DNA content			Mitotic rate			MGC		
BLM	Controls	% of controls	BLM	Controls	% of controls	BLM	Controls	% of controls
2.9±0.3	2.8±0.1	103.6±10.7	8.9±1.5	11.3±1.3	86.7±13.2			
2.9±0.4	2.8±0.5	103.6±14.3	8.6±1.2	9.3±2.3	92.5±12.9			
2.4±0.2	2.0±0.1	120.5±10.0	7.6±1.3	16.9±1.8	45.0±7.7			
3.7±0.3	2.2±0.5	168.2±13.6	6.3±1.4	12.6±1.9	50.0±11.1			
3.9±0.2	2.5±0.5	156.0±8.0	3.4±1.1	7.6±1.0	44.7±14.5			
3.0±0.1	2.7±0.2	113.1±3.7	2.8±0.8	3.3±0.5	84.8±24.2	12.3±1.5	13.7±0.9	89.8±10.9
2.5±0.2	2.7±0.4	89.7±7.4	5.6±1.4	6.8±1.2	82.4±20.6	14.3±0.7	13.7±1.2	95.8±5.1
3.9±0.4	2.8±0.7	137.2±14.3	10.3±1.7	12.8±1.9	80.5±13.3	22.3±0.8	22.2±2.1	100.5±3.6
2.9±0.1	3.6±0.4	80.7±2.8	6.0±0.8	7.8±1.2	76.9±10.3	28.1±1.2	23.0±2.1	121.7±5.2
3.7±0.3	2.6±0.1	142.7±11.5	13.2±0.9	15.9±2.7	83.0±5.7			
3.8±0.2	2.2±0.2	173.6±9.1	10.6±1.1	10.7±1.1	99.1±10.3	21.0±1.1	24.7±4.7	85.0±4.5
2.0±0.2	1.9±0.1	101.3±10.5	14.6±1.7	12.9±1.3	113.2±13.2	19.6±5.2	27.6±2.7	71.0±18.8
2.2±0.4	1.8±0.2	121.4±22.2	7.9±0.6	7.6±1.4	103.9±7.9	28.3±2.6	21.8±0.3	129.8±11.9

licular epidermis. The mitotic rate was expressed as the number of cells entering mitosis per hour per 1000 cells.

RESULTS

Control Values

All animals were exposed to a grip in the neck skin and ip injections every 4 hours for 4 days. These factors explain why the values of the control animals (table 1) are relatively high during the injection period. At Day 9 all control values had reached normal level (table 1). Circadian variations in epidermal cell proliferation exist (13). However, conclusions about BLM effects are only made using percent of control values obtained from animals of the same cage. There is consistent discrepancy between the labeling index (LI) and the proportion of cells with S-phase DNA content (table 1). In general, this indicates that some cells in DNA synthesis phase are not detectable by autoradiography with conventional doses of ^{3}H-TdR (22).

Alterations During the Period of Bleomycin Administration (table 1)

Four hours after the last injection of 12 (Day II), 18 (Day III), and 24 (Day IV) doses of 0.1 mg of BLM, only a slight reduction of the LI was observed. The proportion of cells with S-phase DNA content varied little, with a slightly reduced proportion on Day IV. The proportion of cells with G_2-phase DNA content (fig 1) increased progressively from Day II and reached a maximum of about 170% of control values at the end of the injection period. This accumulation was accompanied by a depression of the mitotic rate to about 45% of control values on Days III and IV (fig 2).

Alterations Subsequent to the Last Bleomycin Injection (table 1)

S-Phase Parameters (figs 1 and 2)

Six hours after the last BLM injection, the LI and the proportion of cells with S-phase DNA content did not differ significantly from control values; the mean grain count (MGC) was slightly reduced. During the next 12 hrs the LI reached a minimum of about 60% of controls, accompanied by a transient drop to about 80% of controls in the proportion of cells in S phase. The MGC did not differ significantly from control level. At 24 hrs the LI again reached control level, accompanied by a parallel change in the relative number of cells in S phase. From Day 2 to Day 6 the LI decreased progressively to about 60% of controls, whereas at Day 6 the proportion of cells with S-phase DNA content increased to a maximum of about 135% of controls. During the same period the MGC decreased to a minimum of about 70%. At the end of the observation period the LI reached control values, whereas the proportion of cells in S phase and the MGC were slightly above control level.

G_2 Phase and Mitotic Rate

After the last injection of BLM the proportion of cells in G_2 phase (fig 3) decreased rapidly and reached control level within 12 hrs. During this time the mitotic rate (fig 4) increased relatively from about 45% of control value at 6 hrs to about 85%. From Day 2 to Day 4 the proportion of cells with G_2-phase DNA content doubled, reaching a peak of

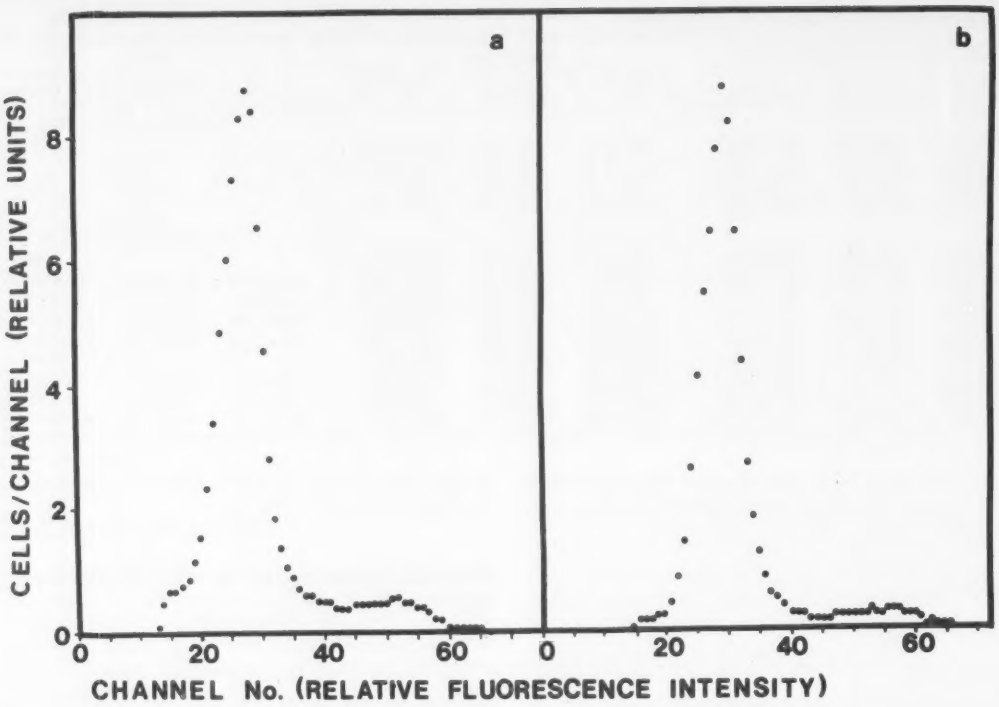

FIGURE 1.—DNA histograms: a. from a BLM-treated animal 12 hrs after the end of the BLM injections and b. from a control showing the decreased proportion of cells with S-phase DNA content.

about 170%, and 2 days later control level was again reached. The mitotic rate showed small variations close to control level during the same time interval.

DISCUSSION

During the period of BLM injections no major inhibitory effect on the LI was seen (table 1). The slight concomitant reduction of cells with S-phase DNA content (table 1) suggests only a slight delay in cell cycle traverse through S phase into G_2 phase. The accumulation of cells with G_2-phase DNA content, accompanied by a low mitotic rate during the same period, indicates that this is due to a block of cell cycle traverse in G_2 phase. This first accumulation of cells with G_2-phase DNA content (Day IV) is probably partially reversible, as indicated by a subsequent rapid fall in the relative number of such cells accompanied by a marked relative increase in the mitotic rate. Therefore, the cells pass in a partially synchronized manner from G_2 phase into mitosis. The mean mitotic rate from Day III to 6 hrs after the last BLM injection was about 50% of controls (0.6/1000 cells per hour). If all of these cells prevented from entering mitosis had been accumulated in G_2 phase, an increase of about 500% would have been expected. However, the peak value was only about 170%. This indicates either a block in the cell cycle progression prior to G_2 phase, increased cell death in G_2 phase, or a combination of both. The fact that the LI and the proportion of cells with S-phase DNA content were only slightly reduced during this period suggests a minor decrease of the cell flux from G_1 to S phase, thereby favoring the assumption of a significant cell loss from G_2 phase, either as lysis in the basal layer or as cell loss caused by tetraploid cells moving into the differentiating cell layer. Even at its maximum the accumulation of cells with G_2-phase DNA content represents only about 4% of the total basal cell population. The release of such a small proportion of cells after cessation of BLM administration cannot lead to a significant degree of subsequent cell synchrony.

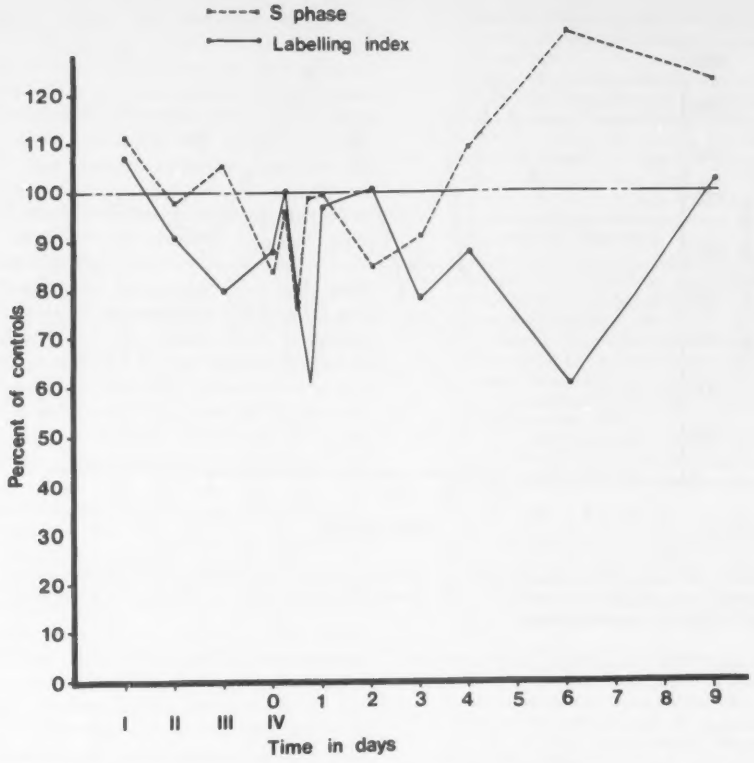

FIGURE 2.—Changes in the proportion of ^{3}H-TdR-labeled cells (LI) and the proportion of cells with S-phase DNA content as % of controls during (Roman Nos.) and after (arabic Nos.) BLM administration.

Shortly after the BLM injections (6–18 hrs), the LI is decreasing, the proportion of cells with S-phase DNA content show a transient drop to about 80% of control values (fig 2), and the MGC is close to control level. This suggests that the cells incorporating ^{3}H-TdR (low LI) do so at an apparently normal rate and that influx to S phase transiently was lower than the efflux. Thus, BLM had an inhibitory effect on cell progression either in G_1 phase or at the G_1/S transition. This is in agreement with previous reports, where higher doses of BLM were used (17). Four days after the end of the BLM administration an accumulation of cells with G_2-phase DNA content again appears, without any significant increase in the mitotic rate. These cells may again represent cells with G_2-phase DNA content unable to proceed further through the cell cycle. Katsumata et al (23) described "transmitotic death" after BLM treatment, and the accumulated cells with G_2-phase DNA content on Day 4 in this study may be of this nature. The moderate absolute value of the G_2-phase accumulation, and the possibility that these cells might be lethally damaged and doomed to die in the following cycle, argue against BLM as a useful drug to obtain synchronized cell cycle traverse in vivo.

The increase of the LI or mitotic rate after cell injury and death in neoplastic tissues may result from recruitment. The proliferative compartment of the hairless mouse epidermis is considered to have a growth fraction close to 1.0 (24). About 30%–40% of the cells in the basal cell layer undergo the initial steps of maturation on the basement membrane before they move to the upper layers and continue differentiation and keratinization. The mean stay of these cells in the basal layer is calculated to be 2.5 days (24).[6] Therefore, in this system with no or few G_0 cells (quiescent cells), re-

[6]Bjerknes R. Model studies of epidermal growth regulation and epidermal carcinogenesis. Thesis. University of Oslo, Norway, 1977.

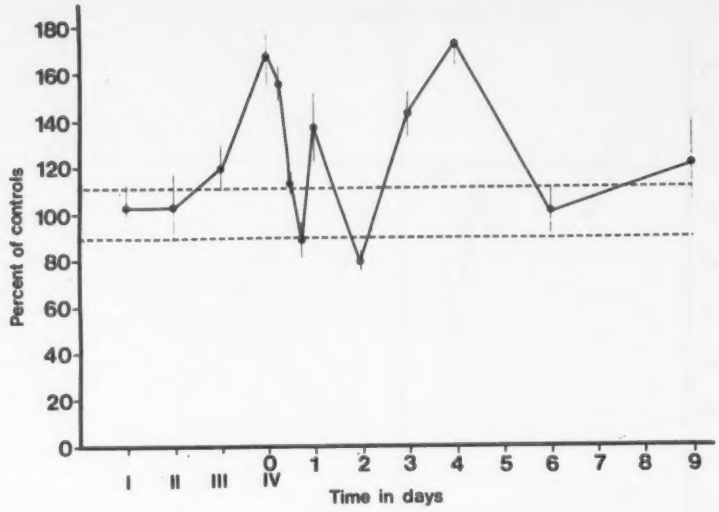

FIGURE 3.—Changes in the proportion of cells with a G_2-phase DNA content as % of controls during (Roman Nos.) and after (arabic Nos.) BLM administration. The distance between the 2 horizontal lines = 2 SEM of 1 control group.

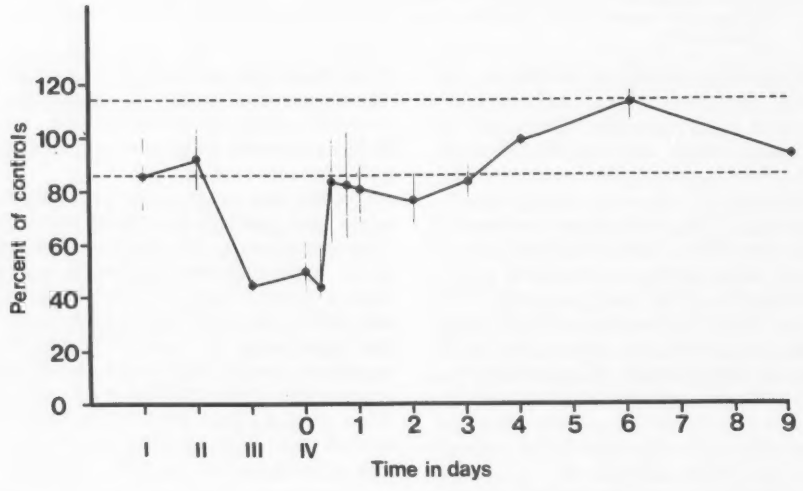

FIGURE 4.—Changes in the mitotic rate determined by accumulation of demecolcine-arrested metaphases plotted as % of controls during (Roman Nos.) and after (arabic Nos.) BLM administration. The distance between the horizontal lines = 2 SEM of 1 control group.

cruitment cannot occur. The duration of the G_1 phase of the proliferating basal cells is calculated to be about 40 hrs of a total generation time of 50–55 hrs (19). A shortening of the G_1 phase, as described during regenerative proliferation (25), would have the same effect on epidermal cell proliferation as recruitment in the system. However, neither the mitotic rate (fig 4) nor the LI exceeded control values after BLM administration indicating that BLM continuously administered prolongs the cell cycle duration in this system.

On Day 6 there is an accumulation of cells with S-phase DNA content, accompanied by low LI and MGC, suggesting that the mean DNA synthesis activity per cell is reduced and probably blocked in some cells. The lack of parallel increase in the relative number of cells with G_2-phase DNA content or in the mitotic rate further supports the belief that this increase does not indicate increased cell proliferation.

The present study has shown that BLM in hairless mouse epidermis does not cause any significant accumulation of cells followed by a synchrony of cell cycle traverse. It confirms previous findings that BLM affects the cell cycle kinetics of the epidermal basal cell layer in a complex way (15–17). Therefore, it is not surprising that a long-lasting administration of a moderate dose of BLM does not act as a good synchrony inducer. In the hairless mouse epidermis the circadian changes in the proportion of cells in the various cell cycle phases are greater than those induced by BLM administration, and might therefore offer a better basis for a synchronization therapy model.

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Phase III Study of ICRF-159 Versus 5-FU in the Treatment of Advanced Metastatic Colorectal Carcinoma[1,2]

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SUMMARY

Thirty-seven previously untreated patients with advanced metastatic colorectal carcinoma were treated in a prospective randomized fashion with either ICRF-159 or 5-FU. The ICRF-159 was administered orally at a dose of 1 g/m²/day for 3 consecutive days every 3 weeks, and the 5-FU was given iv at a dose of 450 mg/m²/day for 5 days every 5 weeks. All patients were evaluated for response and toxic effects after two courses of treatment. All those who failed to meet the criteria for objective response with either a complete remission or a partial response received the other drug in a crossover fashion. Three of the 18 patients (16%) initially treated with 5-FU achieved a partial response while none of the 19 patients initially treated with ICRF-159 achieved a complete or partial response. Nine prior 5-FU-treated patients were crossed over to ICRF-159 and 14 prior ICRF-159-treated patients subsequently received 5-FU. No antitumor response was seen with the secondary agent in this study. The response rate for ICRF-159 (none of 19 patients) predicts that it is unlikely to produce a true response rate of ≥ 20% with a rejection error of < 5%, making it unsuitable as primary therapy for colon carcinoma. The toxicity of 5-FU was moderate and mainly gastrointestinal while the toxicity of ICRF-159 was severe and mainly hematologic.

[Cancer Treat Rep 64:1047–1049, 1980]

Metastatic carcinoma which arises from the colon and rectum continues to present a therapeutic challenge in its refractoriness to conventional chemotherapy. 5-FU is considered the standard against which other agents are evaluated, although response rates for 5-FU have remained a disappointing 15%–20% (1,2). In addition, there is no evidence that the drug contributes to the overall survival of patients with advanced malignant disease. Although responders to chemotherapy appear to have some benefit when compared with untreated patients, the morbidity and inconvenience of receiving frequent iv injections more than offset the small gains in this respect.

The search for more effective treatment therefore continues, and up to this time no single agent or combination of chemotherapeutic agents has a clear advantage over 5-FU used alone (3). This paper summarizes our experience with ICRF-159 used as primary therapy for advanced metastatic colorectal carcinoma in a randomized prospective comparison with 5-FU.

MATERIALS AND METHODS

ICRF-159 is a bis-diketopiperazine which is a nonpolar derivative of EDTA. It was originally synthesized by the Imperial Cancer Research Fund

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facilities in London, England, and has been under investigation for its antitumor properties for a number of years. Although its mode of action has not been elucidated, the drug is lethal to cells passing from the G_2 to the M phase of the cell cycle without inhibiting DNA synthesis (4,5). Animal tumor data reveal a maximum increase in lifespan of 137% against L1210 leukemia when ICRF-159 was given at a dose of 30 mg/day in divided doses (6). In addition, this agent significantly inhibits spontaneous metastasis in mice implanted with Lewis lung carcinoma (7).

Clinical trials in the US indicated that the maximum tolerated dose was 1 g/m²/day given orally in divided doses every 8 hours for 3 days and repeated every 3 weeks (8). Response rates among previously untreated patients (1) and patients previously treated with other chemotherapeutic agents (9) for metastatic colorectal carcinoma were comparable to those for 5-FU used alone. This study is the first randomized prospective comparison of ICRF-159 against 5-FU. In addition, we attempted to determine if 5-FU had any effectiveness against colorectal carcinoma when it was used as a second agent after initial treatment with an investigational drug.

The response criteria used in this study were as follows: *complete response (CR)*—the complete disappearance of all tumor; *partial response (PR)*—a > 50% decrease in the size of all measurable tumors, with no new areas appearing; *stable*—insufficient antitumor response to meet the criteria for PR, with < 25% increase in the size of any lesion; and *progression*—an increase of > 25% in the size of any lesion, the appearance of new metastatic lesions, or decrease in body weight.

Since the purpose of this study was to determine if ICRF-159 was capable of producing objective responses (CR + PR) equal or superior to those produced by 5-FU, all patients who failed to meet the criteria for an objective antitumor response (PR or CR) at the end of two cycles of therapy were crossed over to alternate therapy. Patients who had an objective response and who showed no evidence of life-threatening toxicity continued to receive treatment until progression was evident, at which time they were crossed over to alternate therapy. In order to be evaluated for an antitumor response, all patients were required to receive a minimum of two cycles of chemotherapy.

Prior to chemotherapy and at the completion of a treatment cycle, the following laboratory studies were performed: hemoglobin; wbc, platelet, and differential counts; serum creatinine, bilirubin, alkaline phosphatase, SGOT, calcium, and CEA; and chest roentgenogram. Cbcs and assessment of measurable lesions were obtained every 2–3 weeks.

Patients were randomized to receive (*a*) 5-FU, 450 mg/m², as an iv bolus on Days 1–5 of each 5-week treatment cycle; or (*b*) ICRF-159, 1.0 g/m², orally in divided doses every 8 hours on Days 1–3 of each 3-week treatment cycle.

Side effects and toxicity of both agents were documented and appropriate supportive therapy was done for severe marrow toxicity or stomatitis. Dose modification of the chemotherapeutic agent for those patients who had a CR or PR and who experienced significant toxic effects was as follows: wbc count of > 4000 and platelet count of > 100,000/mm³—100% of dose given; wbc count of 3000–4000 or platelet count of 75,000–100,000/mm³—50% of dose given; and wbc count of < 3000 or platelet count of < 50,000/mm³—no therapy given until counts returned to normal.

RESULTS

Patients were evaluable for toxicity and for response if they completed a minimum of two cycles of treatment while receiving this protocol. Three of the 18 patients (16%) initially treated with 5-FU achieved a PR; none of the 19 patients initially treated with ICRF-159 achieved a CR or PR (table 1). Nine prior 5-FU-treated patients were crossed over to ICRF-159 therapy and 14 prior ICRF-159-treated patients received 5-FU therapy. No antitumor response was seen with the secondary agent in this study.

TABLE 1.—Patient characteristics

Characteristic	No. of patients	
	5-FU group	ICRF-159 group
Total entered	19	20
Total evaluable*	18	19
Females	8	12
Males	10	7
Mean age in yrs (range): 5-FU: 66 (45–75) ICRF-159: 72 (53–82)		
Prior surgery	17	18
Prior radiotherapy	6	4
Prior chemotherapy	0	0
Sites of measurable disease		
Liver	8	9
Lung	8	11
Soft tissue	5	2
Other	1	1

*Of the 2 nonevaluable patients, 1 was lost to followup and 1 died at home, presumably from his metastatic disease, 2 wks after starting therapy. Characteristics described are for the evaluable No. of patients.

TABLE 2.—Toxicity of 5-FU and ICRF-159

Toxic effect	5-FU (18 patients)		ICRF-159 (19 patients)	
	No. of patients	% of patients	No. of patients	% of patients
Nadir wbc counts (cells × 10^3/mm^3)				
3.0–3.4	1	5.5	3	16
2.0–2.9	1	5.5	6	31
1.0–1.9	3	16.6	4	21
0–0.9	0	0	0	0
Nadir platelet counts (cells × 10^3/mm^3)				
75–99	0	0	0	0
50–74	1	5.5	0	0
25–49	0	0	1	5
> 25	0	0	0	0
Gastrointestinal toxicity				
Nausea and/or emesis	3	16.6	10	52
Diarrhea	6	33.2	1	5
Anorexia	0	0	1	5
Mouth ulceration	5	27.7	0	0
Alopecia	2	11	8	42

The toxic effects of the two agents are summarized in table 2. No patient had therapy discontinued or refused therapy because of adverse side effects. A significant proportion of ICRF-159-treated patients experienced side effects: 52% had nausea and emesis, 42% had alopecia, and 68% had hematologic toxic effects manifested chiefly as a depression of the total wbc and absolute neutrophil counts. The 5-FU-treated patients experienced only a moderate degree of toxic effects: 27% had hematologic toxic effects and 33% had diarrhea. No patient had sepsis as a result of leukopenia. Although some patients in the 5-FU-treated group had temporary impairment of nutritional intake due to mouth soreness, none required hospitalization for iv fluid therapy.

DISCUSSION AND CONCLUSION

Because of previous reports that ICRF-159 may be effective in the treatment of colon carcinoma (1,9), this study was done to compare, in a prospective fashion, the effectiveness of ICRF-159 with that of standard therapy (5-FU). The observed response rate for 5-FU (16%) was in the range of responses noted by other investigators (2). ICRF-159 was found to be ineffective or to have such weak antitumor activity against colon carcinoma that it probably does not warrant further trials as primary therapy. Its status in combination therapy remains to be defined. The toxicity of 5-FU given in a standard regimen was moderate, and that of ICRF-159 was considerable. None of the patients who failed to respond to ICRF-159 benefited from treatment with 5-FU given subsequently. It remains to be determined if this reflects tumor refractoriness to the chemotherapy in general or whether pretreatment with an ineffective agent contributes to the refractoriness of colon cancer to standard therapy.

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Phase I Clinical Trial of Spirogermanium[1]

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SUMMARY

Spirogermanium is a new azaspirane antitumor agent, with the metal germanium substituted for a one-carbon moiety in the ring structure. This drug inhibits DNA and RNA synthesis in HeLa cells, is cytotoxic in vitro, and has curative in vivo antitumor activity against the ascitic Walker 256 carcinosarcoma in rats. No hematologic toxicity was recorded during the preclinical toxicologic evaluation. The principal clinical toxic effects observed in this phase I trial were neurologic, manifested as lethargy, dizziness, and ataxia, while a grand mal seizure was produced after an accidental overdose. There was no evidence of hematologic, renal, or hepatic toxicity. A partial response was achieved in a patient with a well-differentiated lymphocytic lymphoma. We recommend that phase II trials be conducted with a twice or thrice weekly dose of 50–80 mg/m², administered in a 30-minute iv infusion.

[Cancer Treat Rep 64:1051–1056, 1980]

Spirogermanium (fig 1) is a newly developed anticancer agent with a novel chemical structure. It is a member of a class of azaspirane compounds which contain a nitrogen linked to a dimethylaminopropyl substituent (1). Spirogermanium also has the metal germanium, a group IVA atom of the periodic table, substituted for a one-carbon moiety in the spirane structure.

Antitumor studies in animals, conducted by Wodinsky and co-workers,[4] have demonstrated that spirogermanium is effective against the ip implanted Walker 256 carcinosarcoma in rats. The optimal dose schedule was 32 mg/kg administered daily on Days 1–9, which produced 500% increased lifespan and 50% 45-day survivors. There were no toxic deaths in normal animals given the same regimen. A moderate inhibition of the 13762 mammary adenocarcinoma and the 11095 prostatic carcinoma in rats was observed in in vivo studies carried out by the Mason Research Institute (Worcester, MA).[4] No antitumor activity has been demonstrated in the L1210 and P388 murine ascitic leukemia models, or against the B16 melanoma.

The preclinical toxicologic evaluation of spirogermanium was performed in CDF_1 mice and beagle dogs under the auspices of the National Cancer Institute program.[5] The LD10 in mice was 35–49 mg/kg (105–147 mg/m²). In dogs, the highest nontoxic dose was 12.5 mg/m² iv, the lowest toxic dose was 25 mg/m², and the lethal dose was 800 mg/m². At lethal doses, generalized seizures were observed in both animal species, occurring immediately after the iv injections. In the dog, 100 mg/m² was the highest dose not associated with central nervous

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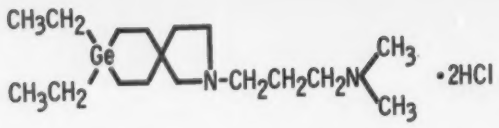

FIGURE 1.—Structure of spirogermanium.

system toxicity, whereas larger doses produced piloerection, nystagmus, and tremors. Additional qualitative toxic effects included focal necrosis of lymphatics, inflammation and necrosis of other gastrointestinal mucosa, and abnormal liver function. There was no evidence of bone marrow toxicity. With these data, we prepared and submitted the IND for spirogermanium in preparation for this phase I trial. The purpose of this clinical study was twofold: (*a*) to define a tolerated dose and the limiting qualitative toxic effects of spirogermanium and (*b*) to determine whether clinical antitumor activity could be demonstrated without myelosuppression, as had been demonstrated in the animal studies.

METHODS

Studies of In Vitro Cytotoxicity in HeLa Cells

HeLa S3 cells were maintained at 37°C in Eagle's minimal essential medium (Grand Island Biological Co, NY). Cell suspensions of 1×10^6 cells/ml were incubated for 2 hours with graded concentrations of spirogermanium. Two hours prior to each time point the cells were pulsed with 1 microcurie of ^{3}H-thymidine (21 curies/mmol) and ^{14}C-valine (270 millicuries/mmol) or ^{3}H-uridine (26 curies/mmol). All isotopes were obtained from the Amersham/Searle Corp, Arlington Heights IL. At all experimental time points, cell aliquots were assayed for viability using trypan blue. Following incubation with the radioisotopes, cells were centrifuged at 1200 rpm for 8 minutes, and the cell pellet was washed with phosphate-buffered saline. DNA, RNA, and protein content of each cell pellet were extracted by the method of Schmidt and Tannhauser (2). DNA concentrations were measured by the diphenylamine method (3), RNA was measured by the orcinol method (4), and protein was measured by the method of Lowry et al (5). The results were expressed as dpm/μg/DNA, RNA, or protein and were compared to the results for control cells.

Clinical Studies

All 35 patients entered in this phase I clinical trial had been previously treated with all accepted forms of chemotherapy for their tumor, to which they had proven resistant. The median age of the study group was 54 years, with a range of 23–74 years. There were 16 men and 19 women. The series included patients with the following tumors: colonic carcinoma (ten), breast cancer (five), non-Hodgkin's lymphoma (four), prostatic cancer (three), head and neck cancer (three), gastric cancer (two), melanoma (two), lung cancer (two), ovarian cancer (one), leiomyosarcoma (one), teratocarcinoma (one), and astrocytoma (one).

All patients had a peripheral wbc count of $> 4000/mm^3$, a platelet count of $> 100,000/mm^3$, and a creatinine clearance > 60 ml/minute; 53% of the patients had a normal SGOT, SGPT, and serum alkaline phosphatase.

The first treatment schedule chosen for study was a single dose of spirogermanium administered once every 4 weeks until there was evidence of either toxicity or tumor progression. As it became apparent that all toxic effects were acute and short-lived, administration once, twice, and thrice weekly was also evaluated. The initial dose tested was 8 mg/m^2, which was one third the toxic dose low in the most sensitive species (beagle dog). Spirogermanium was initially administered by rapid iv injection. With this method, acute neurologic toxicity was evident at the 32-mg/m^2 dose. In order to reduce the severity of these reactions, subsequent higher dose levels were slowly infused over 30 minutes (50–80-mg/m^2 dose range) or over 1–2 hours (120–160 mg/m^2 dose range). In some patients who tolerated a low dose without toxic effects, a higher dose was administered after an interval of observation for possible "carry over" toxicity and therapeutic activity. The doses, schedules of administration, and numbers of courses delivered are summarized in table 1.

Following each treatment, toxicity was monitored weekly by peripheral blood cell counts, serum creatinine, liver function tests, and, in patients receiving the highest doses, by EEG. Partial response is defined as a > 50% reduction in the sum of the tumor area determined by multiplying the largest perpendicular diameters.

RESULTS

Effects on DNA and RNA Synthesis

The results of initial in vitro cytotoxicity studies using HeLa cells in culture are shown in figure 2. Spirogermanium produces a significant inhibition of DNA, RNA, and protein synthesis at concentrations as low as 0.005 mM. Cell viability was 50% at

TABLE 1.—Doses and schedules of administration

Dose (mg/m²)	Schedule	No. of patients	No. of courses
8	Every 4 wks	2	1
16	Every 4 wks	3	1
32	Every 4 wks	4	1,1,1,3
50	Every 4 wks	6	1,1,1,1,1,2
	Weekly	3	3,3,4
65	Every 4 wks	4	1,1,2,3
	Weekly	3	2,3,7
80	Every 4 wks	2	1,2
	Weekly	4	2,2,3,4
	Twice weekly	3	8,9,13
	Every other day × 3, repeated every 3 wks	2	3,5
120	Weekly	5	1,1,1,3,4
160	Weekly	2	1,3

48 hours at the 0.005–0.01 mM drug concentrations. No cells were alive at 24 hours with the 0.015–0.025 mM concentrations.

Clinical Toxicity

Neurologic Toxic Effects

The principal biologic activity was neurotoxicity which was first observed at the 32-mg/m² dose level. When the drug was administered by injection in < 5 minutes, all of three patients experienced mild lethargy immediately after treatment; this drug-related effect resolved rapidly in one patient, but required 12–18 hours for complete reversal in two other patients. A fourth patient treated with a dose of 32 mg/m² administered over 30 minutes experienced no adverse reactions.

The further relationship between the frequency of this transient neurologic toxicity is shown in table 2. At the 50–65-mg/m² dose levels it could be prevented by prolonging the duration of the infusion to 30 minutes. However, at 80 mg/m² and higher doses, this method of administration, extending up to periods of 2 hours, could not completely avoid this complication of treatment. The form in which spirogermanium neurologic toxicity was expressed varied considerably from patient to patient. The principal manifestations were mild dizziness and lethargy, lasting for only a few minutes or several hours. These symptoms were accompanied, in isolated cases, by lateral gaze nystagmus, ataxia, visual distortion, or paresthesias. EEG monitoring during the administration of the 160-mg/m² dose level revealed no changes despite symptoms. All signs of neurotoxicity completely resolved without sequelae, and there was no evidence of cumulative toxicity to the nervous system.

One patient accidentally received ten times the intended dose of 80 mg/m² and developed grand mal seizures during infusion. This patient was a 69-year-old white male with stage D cancer of the prostate that was metastatic to bone, left supraclavicular lymph nodes, and liver; he was previously treated with diethylstilbestrol, orchiectomy, and radiation therapy to painful bone metastases. His past history was remarkable for a cerebrovascular accident 8 years previously, with residual right-sided weakness and hyperreflexia and expressive aphasia. On October 13, 1978, the patient received the first of two weekly doses of spirogermanium (50 mg/m²) as an iv infusion over 30 minutes. No adverse effects were noted. On October 27, 1978, the patient was scheduled to receive a dose-escalation to 130 mg of spirogermanium (80 mg/m²) iv over 30 minutes. After two thirds of the drug had been infused, he was noted to have a tonic-clonic grand mal seizure, with predominantly right-sided activity. The infusion was discontinued, and 4 mg of Valium administered iv with immediate cessation of motor activity. The seizure had lasted approximately 3 minutes. The patient's subsequent neurologic examination disclosed only the previous expressive aphasia and right hemiplegia. Following the seizure, questioning of the personnel involved in preparing the solution revealed that 10 times the prescribed amount had been drawn up for administration, ie, 1300 mg. Of this, the patient actually received 800–900 mg (approximately 550 mg/m²). Over the subsequent 4 weeks, the patient was monitored daily for evidence of neurologic, hematologic, gastrointestinal, hepatic, and renal toxic effects. A computerized axial tomographic scan of the brain disclosed an area of decreased density in the left parietal lobe, which did not enhance with contrast medium, and compensatory hypertrophy of the left lateral ven-

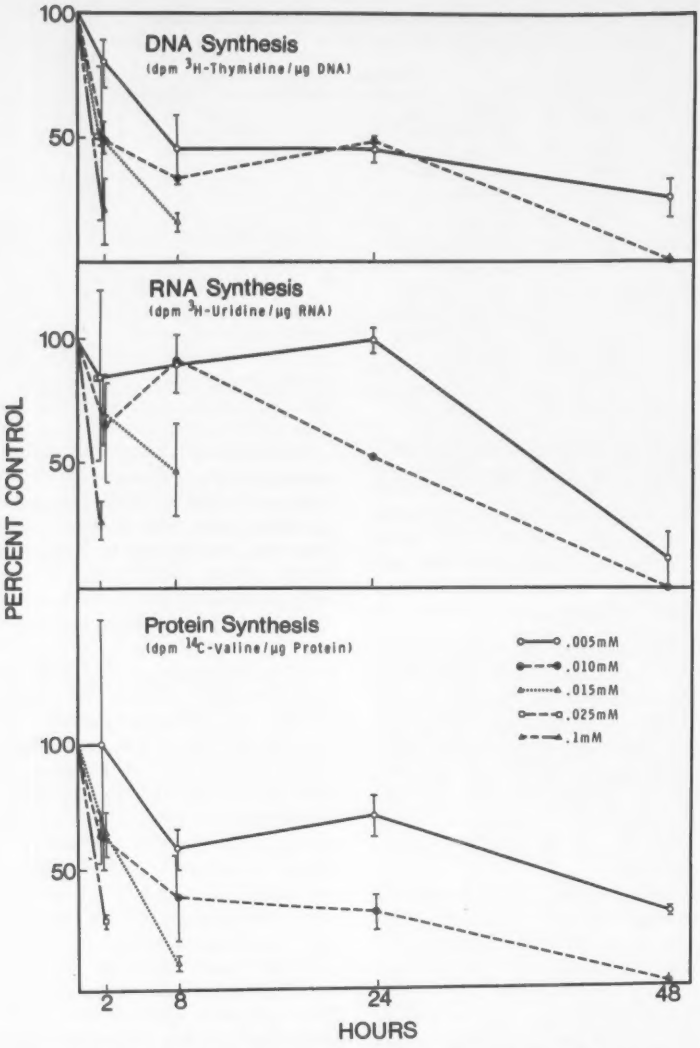

FIGURE 2.—HeLa cell DNA, RNA, and protein synthesis after incubation with spirogermanium.

tricle. This was interpreted as an old infarction consistent with the history of a cerebrovascular accident in 1970. An EEG was also consistent with an old left parietal lobe cerebrovascular accident. On November 7, 1978, the SGOT and SGPT were noted to be slightly above normal. A liver scan showed dramatic enlargement of metastatic disease; an increase in the size of the left supraclavicular lymph nodes and a new scalp nodule were recorded. Wbc counts remained between 5000 and 8000/mm^3 and platelet counts fluctuated between 100,000 and 150,000/mm^3, while serum creatinine, BUN, and bilirubin remained normal during the following 4-week period of observation. The patient's lymph node, skin, and liver metastases continued to enlarge, and he began to show signs of hepatic encephalopathy, with gradual deterioration of mental status until he died on November 24, 1978.

TABLE 2.—Spirogermanium neurologic toxicity: relationship to dose and rate of administration

Dose (mg/m²)	Rate of administration	Total No. of patients	No. of patients with neurotoxic effects
32	Rapid injection	3	3
	30-min infusion	1	—
50	30-min infusion	9	—
65	30-min infusion	7	—
80	30-min infusion	11	4
120	180-min infusion	5	1
160	180-min infusion	2	2

Other Toxic Effects

Patients were monitored sequentially with regard to parameters of hematologic, renal, and hepatic toxicity. There was no evidence of acute or cumulative toxicity. Pain at the injection site and nausea were observed in one patient each at the 50 mg/m² dose level.

Therapeutic Results

One patient with a well-differentiated lymphocytic lymphoma refractory to chlorambucil, prednisone, vincristine, and chlorozotocin achieved a partial response in palpable lymph nodes for 2 months. The dose schedule was 50 mg/m² twice weekly.

DISCUSSION

Spirogermanium is a unique antitumor agent. It is the first azaspirane compound to be used clinically and, in addition to its heterocyclic design, the element germanium is incorporated in the ring structure. The basis for its selection for clinical trials was the demonstration of cytotoxic activity in a malignant cell culture, its curative activity against the in vivo ascitic Walker 256 carcinosarcoma in rats, and the apparent absence of bone marrow toxicity in the preclinical toxicologic studies. The specific mechanism of antitumor activity of spirogermanium, aside from its ability to inhibit synthesis of DNA and RNA, is not known. In our initial studies, we demonstrated an absence of alkylating activity, as measured by the in vitro 4-(*p*-nitrobenzyl) pyridine reaction (6).

The results of this phase I trial were accurately predicted by the toxicologic observations made in mice and beagle dogs. The principal qualitative clinical toxicity was neurologic, taking the form of lethargy, vertigo, and ataxia at moderate dose levels, with grand mal seizures in the patient who received the overdose. The severity of these reactions could be reduced, but not prevented in all cases, by lengthening the duration of administration. While the reactions were unpleasant, they did not result in the discontinuation of treatment in any patients. As many as 13 twice weekly treatments at the 80-mg/m² dose level were tolerated. The animals also accurately predicted that spirogermanium would not be toxic to human bone marrow. No evidence of myelosuppression was found despite measurement of blood cell counts after the overdose of 550 mg/m², nor was there any evidence of cumulative hematologic toxicity after repeated courses at a dose range of 50–120 mg/m².

Only one definite therapeutic response was identified, ie, that in a patient with a diffuse well-differentiated lymphocytic lymphoma which was resistant to treatment with alkylating agents and prednisone. However, this was a phase I study, and all patients had advanced tumors that were refractory to at least one prior form of chemotherapy considered to be the standard for that disease. In addition, evaluable disease was not a requirement for entry in the trial.

We are encouraged by the initial clinical results with spirogermanium, in particular the lack of bone marrow toxicity and the manageable neurologic toxicity. Phase II trials of this agent are now in progress. We recommend that such studies be initiated at a twice or thrice weekly dose of 50–80 mg/m² administered in a 30-minute infusion, with careful attention to possible neurologic toxicity.

In view of the established ability of spirogermanium to produce convulsions in animals and man (albeit at an enormous dose), patients with known seizure disorders and other serious neurologic impairments should be excluded from these trials.

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Phase II Evaluation of Triazinate in Patients With Metastatic Lung Cancer[1,2]

Edward T. Creagan,* Robert T. Eagan, Thomas R. Fleming, Stephen Frytak, James N. Ingle, Larry K. Kvols, and William C. Nichols[3]

Triazinate (TZT), an inhibitor of dihydrofolate reductase, has a broad spectrum of experimental and human antitumor activity. Long-term survivors and some cures have been reported using TZT in rodents with Walker 256 carcinosarcoma (1). In phase I studies, dose-limiting toxic effects were somnolence and dermatitis from both single-dose and 5-day courses repeated at 2–3-week intervals (2,3). Myelosuppression was clinically acceptable and did not seem to be clearly dose-related (3). However, toxicity was related to liver dysfunction. A phase II trial demonstrated objective regressions in patients with lung cancer, particularly adenocarcinoma, and with other tumors (4). A clinically tolerated dose has been 250 mg/m²/day × 3, every 3–4 weeks (5). We conducted a phase II study of TZT at this dose in the treatment of patients with squamous cell (SQC), large cell (LGC), and small cell (SMC) carcinomas of the lung.

MATERIALS AND METHODS

Patients were considered eligible if they had histologic or cytologic evidence of measurable or evaluable disseminated SQC or LGC. Patients with SMC were also entered in the study if clinical or laboratory parameters prevented the use of known effective regimens.

Twenty-eight of 29 patients who were entered in the study were evaluable. One patient with an adenocarcinoma was inadvertently treated in the protocol and was excluded from the analyses. Sixteen patients had received prior chemotherapy primarily with the combinations involving dianhydrogalactitol, doxorubicin (Adriamycin), *cis*-diamminedichloroplatinum(II), and the combination of cyclophosphamide and VP-16-213. Pretreatment patient characteristics are shown in table 1.

TZT was administered as a 1-hour infusion daily for 3 consecutive days at a dose of 250 mg/m² and was repeated every 3–4 weeks. Patients were removed from the study at the first sign of disease progression.

Patients with measurable or evaluable indicator lesions were assessed according to criteria recently reported by Eagan et al (6). Patients who died or who failed to return for treatment were considered to have had disease progression on the date of death or on the day of scheduled return, whichever occurred earlier. No patients were lost to followup.

RESULTS

Therapeutic.—Only three of 28 patients (11%) had an objective regression. One of five patients with LGC who had measurable disease (with prior chemotherapy) had a regression for 29 days and one patient with SMC who had evaluable disease had a regression for 50 days but died from a myocardial infarction. Another patient with evaluable SMC (total, two regressions among eight patients treated) had a regression in the lung after one treatment and then received radiation therapy to the lung lesion per protocol. No regressions were noted among the 15 patients with SQC. Of the 28 patients, disease progression was seen in 12 (40%) during the first 4 weeks following the start of treatment, in an additional seven during the next 4 weeks, and in a total of 24 by 12 weeks. Of the other four patients, two experienced disease progression at 20 and 28 weeks, respectively, one was removed from the study due to toxicity at 3 weeks, and one has stable disease after 27 weeks. None of these four patients had experienced a regression. The 2-, 4-, and 6-month survival probabilities were 89%, 34%, and 29%, respectively, with a mean survival of 3.2 months. The median time to progression and the median survival time for the 12 patients who had no prior chemotherapy were 7 and 17 weeks, re-

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[3]Division of Medical Oncology (*E. T. Creagan, R. T. Eagan, S. Frytak, J. N. Ingle, L. K. Kvols,* and *W. C. Nichols*) and Cancer Center Statistics (*T. R. Fleming*), Mayo Clinic and Mayo Foundation, Rochester, MN.

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TABLE 1.—Pretreatment characteristics of the 28 patients treated with TZT

Characteristic	No prior chemotherapy				Prior chemotherapy				Totals
	SQC	LGC	SMC	Total	SQC	LGC	SMC	Total	
No. of patients	3	1	8	12	12	4	0	16	28
No. of evaluable patients	3	1	5	9	10	3	0	13	22
No. of measurable patients	0	0	3	3	2	1	0	3	6
Age in yrs									
Youngest	71	—	57	57	50	54	—	50	50
Median	75	64	68	69	58	57	—	58	64
Oldest	77	—	77	77	73	60	—	73	77
Sex									
Males	3	0	7	10	11	1	—	12	22
Females	0	1	1	2	1	3	—	4	6
ECOG* PS									
0/1	1	0	4	5	5	1	—	6	11
2/3	2	1	4	7	7	3	—	10	17

*Eastern Cooperative Oncology Group performance score: 0 (fully active)–4 (totally disabled).

spectively. Comparable values for the 16 patients having had prior chemotherapy were 7 and 14 weeks, respectively.

Toxic.—Nausea, vomiting, and diarrhea, the most frequent side effects, were of mild degree in approximately 30% of patients but were moderate-severe in 20%. One third of patients had mild dermatitis which consisted of a blotchy, erythematous, and pruritic rash, especially in the groin. One patient with normal liver function experienced a severe, generalized, intensely pruritic eruption with genital ulcerations. Stomatitis affected ten of 28 patients; this was mild in six patients but moderate-severe in four. Ten patients developed alopecia which was moderate-severe in six. Seven patients (25%) had leukopenia (wbc count $\leq 4100/mm^3$), with nadirs of 1900, 2700, 3000, 3300, 3400, 3500, and 4100 cells/mm^3. These nadirs primarily occurred between Days 7 and 14 following treatment. Two patients had thrombocytopenia (platelet count $< 130,000/mm^3$), with nadirs of 77,000 and 98,000 cells/mm^3 which occurred on Days 14 and 10, respectively.

DISCUSSION

A phase II assessment of TZT was done in patients with advanced bronchogenic carcinoma, many of whom had had prior chemotherapy and impaired performance scores. The overall regression rate was 11%, with antitumor activity in patients with LGC (one of five patients) and SMC (two of eight), but not in patients with SQC (none of 15). This regression rate is quite similar to the 13% reported in patients with adenocarcinoma of the lung (4).

The dose and schedule which we used generated clinically acceptable toxicity in most patients, ample evidence for biologic effect, and a degree of myelosuppression quite consistent with two previous studies which detected leukopenia in approximately 30% of patients (3,4). We were exceedingly reluctant to escalate the dose because persuasive data clearly indicated significant toxicity from doses higher than the one we used. For example, a phase I trial showed that total doses of 500 mg/m^2 produced moderate-severe, and occasionally lethal, toxicity (3). Another phase I study showed that three sequential doses ranging from 200 to 400 mg/m^2 achieved the upper limit of tolerable toxicity (2). In addition, a phase II study using the same regimen as in our trial detected substantial degrees of nausea, vomiting, diarrhea, and stomatitis in approximately 50% of patients. Five patients required hospitalization for complications from treatment and there were two drug-related deaths (5). Therefore, we could not justify exposing our patients to doses in excess of those recommended by previous investigations.

Most patients had disease progression within one or two treatment cycles, and, therefore, we cannot assess the long-term toxicity of TZT. However, the magnitude of the gastrointestinal toxicity and dermatitis might compromise prolonged use of the agent unless downward dose adjustments were made. We can reject, at less than the $\alpha = 0.05$ level of significance, the hypothesis that the objective tumor regression rate is at least 20% among comparable patients with advanced SQC. Additional studies are necessary to define the role of TZT in patients with LGC and SMC.

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Plasma Half-Life and Urinary Excretion of Cyclophosphamide in Children[1,2,3]

N. E. Sladek,* J. Priest, D. Doeden, C. J. Mirocha, S. Pathre, and W. Krivit[4]

SUMMARY

The plasma half-life and urinary excretion of cyclophosphamide were determined in 13 children who had various malignancies and/or who were being prepared for bone marrow transplantation. Disappearance from the plasma following iv infusion over a 1–2-hour period was first-order. Urinary excretion was maximal during the first 8 hours after administration and was essentially complete in 24 hours. The plasma half-life in children not receiving known inducers of hepatic microsomal mixed-function oxygenase activity or cyclophosphamide in the 3-week period prior to each determination ranged from 145 to 390 minutes. These values are lower than those ordinarily found in adult patients. The fraction of the total dose excreted in the urine as the parent compound ranged from 4% to 27%. Repeated administration of cyclophosphamide at approximately 30–60-day intervals did not appear to alter its plasma half-life but did appear to increase its urinary excretion. Daily administration of cyclophosphamide (approximately 50 mg/kg/day × 2 or 4) significantly decreased its plasma half-life and urinary excretion suggesting that it may reversibly induce its own metabolism.

[Cancer Treat Rep 64:1061–1066, 1980]

Cyclophosphamide is an alkylating agent of proven clinical value in the treatment of several neoplasms. Its immunosuppressive properties have been successfully utilized as well. Cyclophosphamide must be activated enzymatically by a hepatic microsomal mixed-function oxygenase system before it can exert its pharmacologic effects. The activities of this system are subject to modulation by numerous factors including age, disease state, and the administration of drugs (1). Mature laboratory animals generally exhibit greater hepatic microsomal mixed-function oxygenase activities than do immature animals (2,3), but the opposite appears to be the case in man (1,4–6).

Several laboratories have investigated the pharmacokinetic properties of cyclophosphamide in adults (7–17); only one has conducted such studies in children, mostly with Burkitt's lymphoma (18). The present investigation was undertaken, therefore, to provide more information about the pharmacokinetic properties of cyclophosphamide in children.

MATERIALS AND METHODS

Patients

Blood and urine samples were collected at various times after the administration of cyclophosphamide (Cytoxan; Mead-Johnson Laboratories, Evansville,

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[2]Supported by Public Health Service grants CA-21737 and CA-07306 (National Cancer Institute) and HL-07145 (National Heart, Lung, and Blood Institute) from the National Institutes of Health, Department of Health and Human Services. J. Priest is the recipient of American Cancer Society Clinical Fellowship 4309.

[3]This is the seventh in a series of papers on cyclophosphamide metabolism. Parts of this investigation appear in abstract form (Proceedings of the American Association for Cancer Research and ASCO 20:159, 1979).

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IN) to nine males and four females ranging from 4 to 18 years of age. The patients had various malignancies (neuroblastoma, osteogenic sarcoma, Burkitt's lymphoma, acute lymphoblastic leukemia [ALL], or acute myelogenous leukemia [AML]) or were being prepared for bone marrow transplantation as a treatment for aplastic anemia, ALL, or AML. All patients with leukemia were in bone marrow remission and patients with other tumors (neuroblastoma, osteogenic sarcoma, or Burkitt's lymphoma) were without gross evidence of disease at the time of cyclophosphamide administration. Cyclophosphamide, at doses ranging from 24 to 60 mg/kg/infusion, was administered iv over 1–2 hours. In all patients, other drugs (table 1) were also administered before and/or during cyclophosphamide infusion and sample collection. On the basis of standard clinical tests, kidney and liver functions were judged to be essentially normal in all subjects.

Analytic Procedure

Venous blood was collected into heparinized syringes and centrifuged to obtain plasma. Urine was collected and centrifuged to remove insoluble components. Plasma and urine samples were stored at –20°C for up to 1 month before analysis. Cyclophosphamide was stable under the conditions of storage.

Extraction of cyclophosphamide from diluted aliquots of the plasma and urine samples was effected with reagent-grade dichloromethane. Extraction efficiency was > 95%. Following extraction, the *N*-trifluoroacetyl derivative of cyclophosphamide was generated as described by Pantarotto et al. (19,20). After derivatization, an internal standard, 2-methylamino-5-chlorobenzophenone dissolved in ethyl acetate, was added and the resultant solution was injected into a gas chromatograph. A known amount of cyclophosphamide added to control plasma or urine and submitted to identical manipulations served as an external standard for each assay.

A Barber Colman Series 5000 gas chromatograph equipped with a flame ionization detector and a Series 8000 Chromocorder was used. The column was a silanized U-shaped Pyrex glass tube (inside diameter, 2 mm; length, 3 feet) packed with 3% OV-1 on 100–120-mesh Chromosorb Q (Supelco, Bellefonte, PA). Prior to its initial use, the column was conditioned according to the manufacturer's instructions. Operating temperatures were: injection port, 220°C; detector, 245°C; and column, programmed from 95°C to 186°C (3 minutes at 95°C and then increased at the rate of 7.5°C/minute up to 186°C). Nitrogen was used as the carrier gas at a flow rate of 35 ml/minute. Air and hydrogen flow rates were adjusted to maximize responses.

TABLE 1.—Other medications

Medication	Code No.
Doxorubicin (Adriamycin)	1
Allopurinol	2
Aluminum hydroxide gel	3
Oxymetholone	4
Atropine	5
Bactrim*	6
BCNU	7
Diphenhydramine	8
Calcium gluconate	9
Chloral hydrate	10
Chlorpromazine	11
Cimetidine	12
Citrovorum factor	13
Codeine	14
Dioctyl sodium sulfosuccinate	15
Compazine	16
Cytosine arabinoside	17
Meperidine	18
DTIC	19
Benzquinamide	20
Erythromycin	21
Cephalexin	22
Furosemide	23
Magnesium citrate	24
Mercaptopurine	25
Methicillin	26
Methotrexate	27
Nystatin	28
Mylanta†	29
Ovral‡	30
Penicillin V potassium	31
Promethazine	32
Phenobarbital	33
Prednisone	34
Carbamazepine	35
Thioguanine	36
Acetaminophen	37
Diazepam	38
Vincristine	39
Viopan-T§	40
Vitamin K	41

*Trimethoprim and sulfamethoxazole.
†Aluminum hydroxide gel, magnesium hydroxide, and simethicone.
‡Norgestrel and ethinyl estradiol.
§High-potency vitamin-mineral formula.

The retention times of 2-methylamino-5-chlorobenzophenone and derivatized cyclophosphamide were 14.7 and 13.5 minutes, respectively. Sharp peaks with minimal tailing were observed for both agents. The minimum detectable amount of derivatized cyclophosphamide was approximately 10 ng/injection.

Areas of response curves were determined by triangulation and utilized to achieve quantitation. Recorder responses were linear to at least 1 μg of derivatized cyclophosphamide injected. Whenever

possible, initial dilutions of the plasma and urine samples were such that 0.1–1 μg of derivatized cyclophosphamide was ultimately injected into the gas chromatograph. The relative accuracy of the method was determined in preliminary experiments by repeatedly assaying (two or three times) 14 different plasma and urine samples for their cyclophosphamide content, each time relative to a separately prepared external standard; a coefficient of variation of 7.96% was obtained.

RESULTS AND DISCUSSION

Disappearance of cyclophosphamide from the plasma following its iv infusion over 1–2 hours was first-order (fig 1). Plasma half-lives in children not receiving known inducers of hepatic microsomal mixed-function oxygenase activity (patients 1-12) or cyclophosphamide in the 3-week period prior to each determination ranged from 145 to 390 minutes (table 2). DeVita and Adamson (18) reported similar half-lives in children receiving the same or smaller doses of cyclophosphamide. The plasma half-life of cyclophosphamide in children appears to be somewhat shorter than that in adults where it is usually about 4–10 hours (7–18), but considerably longer than that in tumor-free and tumor-bearing experimental animals where it is < 1 hour (N. E. Sladek, unpublished observations; [18–22]). Since the removal of cyclophosphamide from the blood is largely

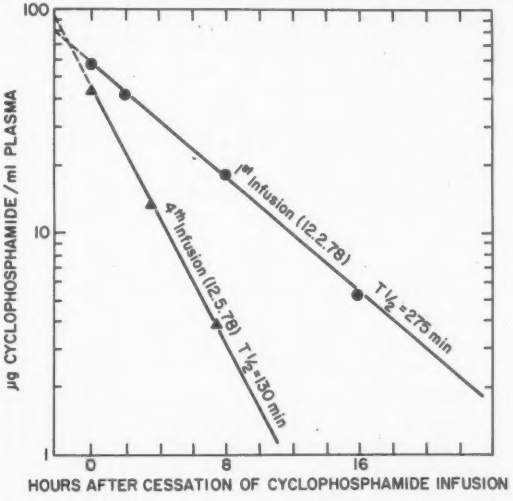

FIGURE 1.—Plasma half-life of cyclophosphamide in patient 7. The patient received cyclophosphamide by iv infusion over a 1–2-hr period at 24-hr intervals, 53 mg/kg/infusion × 4. Blood samples were collected and analyzed for cyclophosphamide content as described in Materials and Methods.

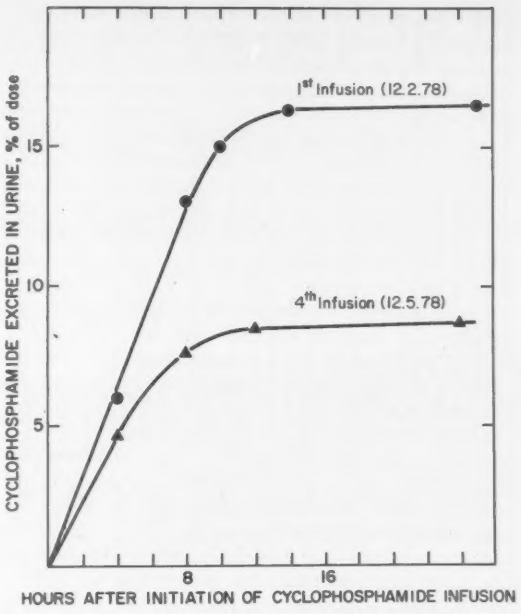

FIGURE 2.—Urinary excretion of cyclophosphamide in patient 7. The patient received cyclophosphamide by iv infusion over a 1–2-hr period at 24-hr intervals, 53 mg/kg/infusion × 4. Urine samples were collected and analyzed for cyclophosphamide content as described in Materials and Methods.

mediated by hepatic microsomal mixed-function oxygenase, these observations are in agreement with reports from other laboratories (1,4–6) that the activity of this enzyme system is greater in children than in adults.

The apparent volume of distribution ranged from 0.50 to 0.84 liter/kg, indicating that cyclophosphamide distributes in total-body water (table 2). Similarly, and consistent with this indication, the mean value in adults is approximately 0.55 liter/kg with a range of 0.32–1.90 liters/kg (7,10,11,14,17).

Urinary excretion of unchanged cyclophosphamide was essentially complete in 24 hours, and the rate of urinary excretion was maximal during the first 8 hours after the initiation of the infusion (fig 2). Four percent to 26% of the dose was excreted unchanged in the urine (table 2). Similar observations have been made in children, adults (7–13,15,16,18), and experimental animals (N. E. Sladek, unpublished observations; [19,20]).

There were no obvious differences in plasma half-lives or urinary excretion with respect to diagnosis, other antitumor medication, or clinical status at the time of cyclophosphamide infusion (table 2). In a

TABLE 2.—Plasma half-lives and urinary excretion of cyclophosphamide in children

Patient No.	Sex	Age (yrs)	Weight (kg)	Diagnosis	Other medications: Before study*	Other medications: During study†	Cyclophosphamide: Dose interval (days)‡	Cyclophosphamide: Date of infusion (mo/day/yr)	Cyclophosphamide: Dose (mg/kg)	Cyclophosphamide: Plasma half-life (mins)§	Cyclophosphamide: Vd (liter/kg)¶	Cyclophosphamide: % in urine**
1	F	18	79	AML	6,10,14,15,22,28, 30,31,37,38	6,15,23,28,41	22	5/27/78	60	190	0.51	—
							1	5/28/78		155	0.50	—
2	F	4	16	ALL	None	11,18,23,28,32	—	11/08/78	60	—	—	16.6
							1	11/09/78		—	—	6.0
3	M	14	38	ALL	10,18,28,37	23	37	11/10/78	60	—	—	17.7
							1	11/11/78		—	—	11.1
4	F	16	51	ALL	6,10,25,28,34	6,23,25,28,34	21	1/12/79	60	145	0.58	20.3
						6,15,23,28,34	1	1/13/79		90	0.59	11.4
5	F	16	55	AML	17,36	8,16,23,38	—	3/22/79	60	285	0.71	16.6
							1	3/23/79		130	0.73	14.2
6	M	10	35	Aplastic anemia	9,10,14,15,26	23	—	6/02/78	53	205	0.66	—
							1	6/03/78		—	—	—
							1	6/04/78		—	—	—
							1	6/05/78		135	0.60	—
7	M	8	23	Aplastic anemia	4,10,28,31,34	20,23	—	12/02/78	53	275	0.81	16.5
							1	12/03/78		—	—	8.0
							1	12/04/78		—	—	7.3
							1	12/05/78		130	0.84	8.7
8	M	18	60	Burkitt's lymphoma	13,14,18,27,31	7,17,20,23,28	23	12/09/78	56	320	0.67	16.3
						7,16,17,20,23,28	1	12/10/78		—	—	12.3
						7,8,16,17,20,23,28	1	12/11/78		—	—	8.1
						20,23,28,32	1	12/12/78		115	0.67	6.9
9	M	14	68	Neuroblastoma	None	19,39	35	3/01/78	25	—	—	3.9
					None	None	27	3/28/78		360	0.61	3.6
					None	11,19,39	27	4/24/78		390	0.58	8.3
					19	19,39	32	5/26/78		330	0.74	—
					19	39	46	7/11/78		360	0.66	10.3
					19	39	31	10/16/78		335	0.68	—
10	M	14	47	Osteogenic sarcoma	None	11	49	2/09/78	45	—	—	17.7
					1	16	56	4/06/78	50	270	0.83	26.2
					11,13,27	11,20	42	5/18/78		300	0.76	—
					None	11	57	7/14/78		320	0.81	—
11	M	7	25	Burkitt's lymphoma	2,3,9,12,15,24,34, 38,40	None	2	3/31/78	24	—	—	12.4
							2	4/02/78		160	0.62	12.1
					13,14,17,21,27,34		46	5/18/78		240	0.69	—
12	M	14	28	Burkitt's lymphoma	2,6,15,34,39	2,6,15,34	30	5/24/78	26	145	0.68	—
					2,6,12,15,23,29,34, 39,40	2,5,6,12,18,29,32,34	2	6/01/78		140	0.60	—
13	M	11	48	Osteogenic sarcoma	33	20,23,33	77	4/25/78	52	65	0.65	4.0
					34	20,23,35	51	12/13/78	37	—	—	5.3

*Includes all other drugs administered during the 2 wks preceding the initiation of each cyclophosphamide infusion; *see* table 1 for numerical code.

†Includes all drugs administered within the first 24 hrs after initiation of each cyclophosphamide infusion; *see* table 1 for numerical code.

‡Days since cyclophosphamide was last given; dash indicates that cyclophosphamide was never previously given.

§3–8 data points were used to obtain each value; $r \geq 0.98$ in all cases.

¶Volume of distribution. Equal to dose of cyclophosphamide/C_o. C_o obtained by extrapolation of log-linear slope (fig 1) to 0-time (taken to be the time at which one half of the infusion had been completed).

**Percent of total dose appearing in urine as cyclophosphamide in the first 24 hrs after initiation of each cyclophosphamide infusion.

patient receiving phenobarbital for seizures (No. 13), the plasma half-life of cyclophosphamide was 65 minutes; the fraction excreted in the urine of this patient was small but within the normal range. Barbiturate pretreatment is known to increase the rate at which cyclophosphamide is metabolized by hepatic microsomal mixed-function oxygenases of animals (21–24). Some investigators have found that barbiturate pretreatment causes a decrease in the half-life of cyclophosphamide in man (7,8,23); others have not (18).

In patients receiving repeated infusions of cyclophosphamide at approximately 30–60-day intervals (Nos. 9 and 10), the plasma half-life and apparent volume of distribution did not vary appreciably from infusion to infusion (table 2). Mouridsen et al (10,15) reported similar findings when cyclophosphamide (0.02–10 mg/kg/dose) was administered to adults with cancer at 2–14-day intervals. The fraction of the administered dose excreted in the urine appeared to increase with each successive treatment (table 2).

Daily administration of cyclophosphamide (50–60 mg/kg) for 2 (patients 1–5) or 4 (patients 6–8) consecutive days resulted in a decrease of its plasma half-life (table 2 and fig 1). Since urinary excretion of cyclophosphamide also decreased (table 2 and fig 2), the decrease in plasma half-life cannot be explained by increased urinary excretion. The apparent volume of distribution was not altered in these experiments. The only other drug that was always administered to these patients before or during cyclophosphamide therapy was furosemide. Administration of furosemide did not decrease the half-life of cyclophosphamide in rats (N. E. Sladek, unpublished observations) nor does it appear to do so in adults (9).

The reversible nature of the cyclophosphamide-induced decrease in its own plasma half-life is suggested by the data obtained for patient 11. The data obtained for patient 12 suggest that there is a minimum value beyond which the plasma half-life of cyclophosphamide cannot be lowered by repeated administration of the drug at short intervals. Neither of these patients received furosemide.

These findings suggest that cyclophosphamide reversibly induces its own metabolism. In agreement with our observations, Bagley et al (9) reported that in adults receiving relatively low doses (6.8–12 mg/kg) of cyclophosphamide for 5 consecutive days, the plasma half-life of cyclophosphamide was shorter at the end of therapy; some of their patients did not receive furosemide or any other drugs before or during cyclophosphamide therapy. D'Incalci et al (17) also found that the half-life of cyclophosphamide was shorter at the end of treatment in adults receiving it daily (about 1–2 mg/kg/day) for 6–13 months; their patients did not receive furosemide or any other drugs. In contrast to our findings, they found a 50% decrease in the apparent volume of distribution at the end of therapy.

Some investigations do not support the contention that cyclophosphamide induces its own metabolism. Mouridsen et al (15) did not observe a significant decrease in the plasma half-life of cyclophosphamide after administering it for 13–33 consecutive days to adults at daily doses of 2 mg/kg. Moreover, cyclophosphamide administration (40 mg/kg) did not increase hepatic microsomal mixed-function oxygenase activity, as judged by the aminopyrine breath test, in adults with cancer (25). Several investigators have reported that administration of cyclophosphamide to tumor-free animals decreases or does not significantly alter hepatic metabolism of cyclophosphamide and other drugs (21,24,26–30). Morphologic changes in hepatocytes following cyclophosphamide administration to rats have also been reported (31).

Perhaps relevant to our observations are the reports that hepatic microsomal mixed-function oxygenase-catalyzed metabolism of cyclophosphamide and other drugs is generally depressed in tumor-bearing animals (32,33), and that although cyclophosphamide does not induce its own metabolism in tumor-free rats, it does so in tumor-bearing rats (21). Little is known about the relative activity of this enzyme system in cancer patients and cancer-free "normal" human subjects. Hepner and co-workers (34,35) reported that hepatic microsomal mixed-function oxygenase activity was depressed, as judged by the aminopyrine breath test, in patients with malignant disease that included metastatic involvement of the liver. Higuchi et al (36), utilizing antipyrine half-life as an indicator, reported depressed hepatic drug metabolizing activity in patients with disseminated gastric carcinoma. On the other hand, antipyrine half-lives in patients with localized gastric carcinoma (36) or lung cancer (37) were only slightly (and not signficantly) longer relative to those in control subjects. It should be noted that all of our patients were in remission or were being treated for aplastic anemia.

If cyclophosphamide is inducing its own metabolism in our patients, it is doing so relatively rapidly, ie, within 24 hours. Substantial induction, by classic inducing agents such as phenobarbital, of the hepatic microsomal mixed-function oxygenase system that metabolizes cyclophosphamide in rats (21,38) takes longer (39).

Whatever the mechanism, the therapeutic and toxic consequences, if any, of a shorter cyclophos-

phamide plasma half-life following its repeated daily administration are unknown.

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Phase I Trial of *N*-(Phosphonacetyl)-L-aspartic Acid (PALA)[1]

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SUMMARY

***N*-(Phosphonacetyl)-L-aspartic acid (PALA) was given as a 5-day continuous infusion in a phase I trial. Dose-limiting toxic effects noted were diarrhea occurring at doses of ≥ 6 g/m²/course, mucositis occurring at doses of ≥ 7.5 g/m²/course, and skin rash occurring at doses > 9 g/m²/course. No significant CNS, hemorrhagic, gastrointestinal, or hematologic toxicity was noted. In patients with measurable tumor volume, no significant antitumor responses were seen. A dose of 9 g/m²/course is recommended for a phase II trial.**

[Cancer Treat Rep 64:1067–1071, 1980]

N-(Phosphonacetyl)-L-aspartic acid (PALA) is a transition-state analog of the enzyme, aspartate transcarbamoylase (ATCase), which mediates a primary step in the de novo pyrimidine synthesis pathway. As a specific transition-state analog for the ATCase reaction, PALA derives its principal biochemical activity through inhibition of the production of both orotic acid and uridine from carbamoyl-L-aspartate. PALA has been shown to be a potent competitive inhibitor of aspartate transcarbamoylase in *Escherichia coli* (1), mouse spleen (2), and SV40-transformed baby hamster kidney cell models (3). In mouse salivary gland tissue, PALA has been shown to completely inhibit isoproterenol-induced DNA synthesis (4). In mice, the simultaneous administration of uridine or carbamoyl aspartate blocked both the toxicity and the antitumor effect of PALA. Specifically, carbamoyl-DL-aspartate is more effective in blocking the antitumor activity of PALA, while uridine administration more completely prevents toxicity than antitumor effect (5).

In animal models, PALA has been reported to have a spectrum of antitumor activity quite different from that of other antimetabolite tumor agents. PALA is more effective against tumors with slower doubling times and is least effective against faster growing tumor models. PALA was capable of producing an increase in lifespan of up to 86%, with no complete responses when tested against ip B16 melanoma. Early ip administration of PALA in mice bearing sc inoculated Lewis lung carcinomas produced complete responses in 50% of subcutaneous tumors. In addition, PALA has shown antitumor activity against advanced tumors produced by iv inoculation of Lewis lung carcinoma in mice. Activity has also been noted in glioma 26, colon adenocarcinoma 26, and C3H mammary carcinoma (6).

In preclinical large animal toxicity studies, gastrointestinal disturbances and CNS effects were dose-limiting toxic effects of PALA administration. In both dogs and monkeys, marked anorexia, weight loss, diarrhea, and hematochezia were common. CNS toxicity including unconsciousness, convulsions, ataxia, and death were associated with bolus administration of PALA. Mild lymphopenia in dog trials was the only hematologic abnormality associated with PALA administration. Mild liver function abnormalities were seen in large animals and in mice at sublethal dose levels.[4]

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[4]Preclinical Animal Toxicity Report. Prepared by the Laboratory of Toxicology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, 1977.

A number of preclinical trials have been carried out to test the schedule dependency of the effects of PALA. In active tumor model systems, only slight dependency could be elicited, with prolonged treatment schedules superior to single-dose schedules with respect to antitumor effect (6).

The rapid clearance of PALA via renal excretion and the avoidance of CNS toxicity associated with PALA administered by bolus therapy[5] suggested that a constant infusion of PALA would be appropriate. The results of a phase I clinical trial of PALA given as a 5-day continuous infusion are the subject of this report.

MATERIALS AND METHODS

Patients with histologic evidence of advanced malignancy refractory to conventional therapy, or for whom no proven therapy was available, were included in this study. Most of the patients (table 1) entered in our clinical trial had a performance status of < 2 (> 50% ambulatory) and an estimated survival time of at least 3 months. No patient had received radiotherapy or chemotherapy for at least 30 days prior to the PALA administration. All patients had acceptable hematologic parameters (wbc count > 4000/mm^3 and platelet count > 150,000/mm^3) and normal hepatic function. Twenty-eight of the 29 patients entered in this study had a creatinine clearance of > 50 ml/minute.

PALA was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, and was prepared in 1-g ampules reconstituted in 10 ml of 0.85% normal saline without preservative. The pH of the ampules was 6.5–7.5. Prior to administration of the drug, the daily dose of PALA was mixed in 0.85% normal saline to a volume of 1000 ml. This volume was given as a constant infusion daily for five equal daily doses. Courses of treatment were repeated at 3-week intervals. Treatment was delayed in patients having residual gastrointestinal or dermatologic symptoms. The phase I trial was initiated at a total dose level of 750 mg/m^2/course.

In the absence of dose-limiting toxicity, the dose level was escalated (table 2). Dose escalation was initially carried out at 750-mg/m^2/course increments. However, following preliminary reports of other investigators (*see* footnote 5), dose escalation was subsequently carried out at 1500-mg/m^2/course increments until dose-limiting toxicity was reached.

[5]Minutes of the meeting of the Phase I Working Group, Oct 18, 1978. Prepared by the Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	29
Mean age in yrs: 45.7	
Performance status	
>50% ambulatory	24
<50% ambulatory	5
Previous therapy	
Chemotherapy	25
Radiotherapy	1
Radiotherapy and chemotherapy	2
No therapy	1

Prior to the beginning of chemotherapy, all patients had routine complete history and physical examination. Initial laboratory evaluation included cbc with differential and platelet counts, serum electrolytes, glucose, liver function tests, and baseline creatinine clearance. Where appropriate, measurable disease was fully documented. Weekly followup studies included cbc with differential and platelet counts, liver function tests, BUN, and creatinine. Creatinine clearance was repeated prior to each course of chemotherapy.

Response criteria were defined as: *partial response*—a > 50% decrease in all measurable disease for > 1 month; *stable disease*—a < 25% change in tumor size stable over 1 month; and *disease progression*—an objective increase of > 25% of existing disease or the appearance of new tumor lesions.

RESULTS

The three major dose-related toxic effects noted were diarrhea, mucositis, and skin rash (table 2). Diarrhea was the most common and severe of the side effects associated with constant-infusion PALA. It typically occurred from Day 4 to Day 10 of the PALA course, with a median duration of approximately 6 days; dose-limiting episodes lasted as long as 3 weeks. Dose-limiting diarrhea was often associated with midabdominal cramps, anorexia, and a decrease in performance status. In contrast to other phase I studies which utilized bolus administration, there was no associated hematochezia or hemorrhagic proctitis. The diarrhea was clearly dose-related (table 3), with most patients who received 6 g/m^2/course of PALA having significant gastrointestinal toxic effects. Dose-limiting gastrointestinal toxicity occurred at the higher doses, with the maximum tolerated dose being approximately 9 g/m^2/course.

The second major toxic effect associated with constant-infusion PALA was mucositis. The mucositis

TABLE 2.—Dose-limiting toxic effects of PALA

Dose (mg/m²/day)*	No. of patients	% of patients developing dose-limiting toxic effect		
		Diarrhea	Mucositis	Skin rash
150	5	0	0	0
240	4	0	0	0
600	5	20†	0	0
750	3	0	0	0
900‡	7	14	14	0
1200	5	25	25	0
1500	8	38	13	0
1800	12	50	17	17
2000	5	100	60	20

*Course of therapy consisted of 5-day infusion at daily doses.
†Creatinine clearance < 27 ml/min.
‡Dose escalation changed from 750 to 1500 mg/m²/course.

typically occurred from Day 3 to Day 10 of the PALA course, with a median duration of 7 days. Mucositis appeared as a dose-limiting toxic effect in patients receiving the highest dose level of PALA; most patients tolerated 9 g/m²/course of PALA without dose-limiting mucositis (table 3).

The third major toxic effect was skin rash. A characteristic erythematous plaque appeared on the intertriginous, neck, and skin-fold areas approximately 6 days after the start of PALA administration. There were two episodes of diffuse erythroderma. Histologically, the skin rash represented a toxic necrolysis of the basement membrane region, without polymorphonuclear leukocyte infiltrate or hypersensitivity phenomena. There was no elevation of antinuclear antibody titer or erythrocyte sedimentation rate. The skin rash appeared as a dose-limiting toxic effect (table 3), but only at the highest dose levels.

Hematologic, hepatic, renal, or CNS toxicity was not observed in any patient who was entered in our study.

All of the 29 patients entered in our study were evaluable for toxic effects of constant-infusion PALA. Dose-limiting toxicity of any type was unusual with a dose of < 6 g/m²/course. At a dose of 9 g/m²/course, approximately 50% of patients experienced some dose-limiting toxic effect, usually diarrhea.

Fifteen of the 29 patients who received PALA doses of ≥ 6 g/m²/course in our study were evaluable for antitumor effect. All patients were available for evaluation of antitumor effect for at least 3 months following the initiation of PALA therapy. There were no significant tumor responses in the 15 evaluable patients. There was stabilization of disease in two patients with sarcoma metastatic to the lung (3 months), stabilization of disease in one patient with poorly differentiated adenocarcinoma of the lung (4 months), and stabilization of disease in one patient with abdominal mesothelioma (3 months) (table 4).

DISCUSSION

In our study the effects of constant-infusion PALA were studied using a 5–7-day continuous infusion at 3–7-week intervals. The maximum tolerated dose on this regimen was thought to be 9 g/m²/course. The toxic effects were dose-related, reversible, and confined to diarrhea, mucositis, and skin rash. These toxic effects are similar to those noted in other studies using a 5-day bolus infusion schedule (7). CNS toxicity was not observed, suggesting some schedule dependency for this potentially life-threatening effect. Hematologic toxicity was not observed. The results of this study confirm that there is no significant schedule dependency for PALA toxicity, since our dose-limiting toxic effects occurred at a dose of PALA similar to the maximum tolerated dose suggested by other phase I trials (*see* footnote 5). Patients with impairment of creatinine clearance to < 50 ml/minute should have dose reduction to approximately 50% of the maximally tolerated dose.

To date, we have observed no significant antitumor response in our study group. Interestingly, we did observe stabilization of disease in two of three patients with high-grade soft tissue sarcoma metastatic to the lung.

Constant-infusion PALA at a dose of 9 g/m²/course results in toxicity to gastrointestinal mucosa without bone marrow or serious CNS toxicity. This may be related to differential requirements for long-term exposure to PALA among normal tissues for the appearance of cytotoxicity. For phase II trials a constant-infusion schedule can be recommended,

TABLE 3.—PALA toxicity

Dose (mg/m^2/day)	No. of patients	No. of patients		
		Toxicity degree		
		None	Mild	Dose-limiting
		Diarrhea		
150	5	5	0	0
240	4	4	0	0
600	5	5	0	1*
750	3	3	0	0
900	7	5	1	1†
1200	5	2	2	1
1500	8	2	3	3
1800	12	2	4	6
2000	5	0	0	5
		Mucositis		
150	5	4	0	0
240	4	4	0	0
600	5	5	1	0
750	3	3	0	0
900	7	5	1	1†
1200	5	3	2	1†
1500	8	3	4	1
1800	12	3	7	2
2000	5	0	2	3
		Skin rash		
150	5	4	0	0
240	4	4	0	0
600	5	6	0	0
750	3	3	0	0
900	7	6	1	0
1200	5	3	2	0
1500	8	3	5	0
1800	12	4	6	2
2000	5	0	4	1

*Creatinine clearance < 27 ml/min.
†Creatinine clearance 47 ml/min.

TABLE 4.—Antitumor activity of PALA (15 evaluable patients)

Disease	No. of patients	Response (No. of patients)
Adenocarcinoma, colon	4	No response
Sarcoma	3	Stable × 3 mos (2)
Adenocarcinoma, breast	3	No response
Mesothelioma	1	Stable × 3 mos (1)
Diffuse poorly differentiated lymphoma	1	No response
Medullary carcinoma, thyroid	1	No response
Undifferentiated carcinoma, lung	1	Stable × 4 mos (1)
Melanoma	1	No response

based on the lack of observed CNS side effects as well as the potential for achieving prolonged elevated serum PALA levels which may be necessary for the production of cytotoxicity in vivo (8).

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Danazol Treatment of Advanced Breast Cancer[1]

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SUMMARY

Danazol, an effective agent in the treatment of benign breast disease, has been administered to 41 patients with advanced breast cancer. These patients were treated with danazol for periods ranging from 1 to 40 weeks and responses were seen in seven of 41 (17%) patients. No responses were seen in the four premenopausal patients treated. Side effects were seen in nine of 41 (22%) patients, but in most patients these stopped when the dose of danazol was reduced. This preliminary study indicates that danazol may be an effective form of hormone treatment in patients with advanced breast cancer.

[Cancer Treat Rep 64:1073–1076, 1980]

Danazol is a synthetic steroid that inhibits luteinizing-hormone (LH) and follicle-stimulating-hormone (FSH) release by the pituitary and possesses impeded androgenic and antiprogestogenic activity (1). It also has a direct inhibitory effect on steroidogenesis (2).

These mechanisms presumably account for its effectiveness in benign breast disorders (3) and in rat mammary tumors, 66% of which regress under treatment (4).

We have therefore carried out a study to assess the effect of danazol in patients with breast cancer.

PATIENTS, MATERIALS, AND METHODS

Danazol was administered to 41 female patients at the Medical Breast Unit, Royal Marsden Hospital. All had histologically proven metastatic breast carcinoma.

Five of these patients were terminally ill, two with liver failure and three with progressive bone and lung disease. All five patients died of progressive disease; they had received the drug for < 3 weeks and were therefore unevaluable. Two patients failed to take the treatment as prescribed. The remaining 34 patients have received the drug for periods ranging between 3 weeks and 10 months. Twenty of the 34 patients had not received prior systemic therapy for metastatic breast cancer. The remaining 14 patients had received prior endocrine therapy or chemotherapy, but no treatment had been given within 3 weeks of starting treatment with danazol.

Ten patients had accessible skin nodules that were biopsied and immediately frozen in liquid nitrogen for measurement of estrogen receptor (5). Sensitivity of this assay was 10 femtomols/mg of cytosol.

Full staging (physical examination, chest roentgenogram, skeletal survey, bone scan, liver function tests, and hydroxyproline excretion) was done before and after 6–9 weeks of treatment, unless there was clear evidence of disease progression with worsening of symptoms after 3 weeks of ther-

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TABLE 1.—Response by site in 34 patients who received danazol for at least 3 wks (34 patients)

Response	No. of overall responses (%)	No. of responses by site		
		Bone	Visceral	Soft tissue
Complete or partial	7 (20.6)	0	2	5
Disease stabilization (> 2 mos)	4 (11.8)	6*	0	3
Progressive disease	23 (67.6)	14	15	10

*All patients had relief of pain.

apy, in which case reassessment was carried out at that time (seven patients). Assessment of response was made using UICC criteria (6). For soft tissue lesions, the sum of the products of the diameters of each lesion should decrease by ≥ 50%. For undimensional lesions, response was defined as a decrease of ≥ 50% in this dimension. Recalcification or healing of osteolytic lesions was interpreted as a response in bone. A response, as defined, had to be maintained for at least 2 months. Serum LH and FSH were measured by radioimmunoassay, using MRC 69/104 standard as previously described (7,8). LH and FSH were measured before and 6–9 weeks after therapy in nine patients.

Danazol was started at a dose of 100 mg 3 times daily for the first week, and was then increased to 200 mg 3 times daily until relapse or disease progression if no untoward side effects occurred. The dose is similar to that used in benign breast disorders (3).

RESULTS

Two patients achieved a complete response and five achieved a partial response to treatment; six of these patients are continuing in remission for 3-10 months after the start of therapy. Sites of response in 34 patients who received treatment for at least 3 weeks are shown in table 1. Responses occurred in five of the 18 soft tissue disease sites and in two of the 17 visceral sites. No healing is yet apparent of any skeletal lesion, although two of the seven patients who showed responses and three of the four patients who showed disease stabilization in other sites (table 1) had subjective pain relief with no evidence of progression of skeletal metastases. The response rate was 26% for the 27 patients who received therapy for ≥ 6 weeks.

Table 2 shows some of the characteristics of the responders and nonresponders. The mean age of responders was somewhat higher than that of nonresponders and none of the four premenopausal patients showed objective evidence of response. Only one of the three tumors obtained from the responders showed significant binding of tritiated estradiol. Neither of the patients with receptor-negative tumors had received prior therapy with antiestrogens. Thirteen patients did not receive the full dose of 600 mg because of side effects (nine patients) or previous cardiac disease (four patients),

TABLE 2.—Danazol treatment: characteristics of responders and nonresponders (total, 41 patients)

	Responders (7 patients)	Nonresponders (34 patients)
Mean age in yrs (range)	69.7 (47–83)	62.3 (34–83)
Mean time receiving therapy in mos (range)	6.1 (3–10)	1.7 (0.25–6)
Menopausal status*		
Premenopausal	0/4	4/4
Postmenopausal	7/37	7/37
Positive estrogen-receptor status*	1/3	2/7
Mean tumor-free interval in mos (range)	31.6 (1–96)	23.0 (0–120)
Reduced dose (300 mg)*	3/7	10/34
Previous systemic treatment*	1/7	17/34

*Values = No. of patients with characteristic/total No. of patients treated.

but this did not appear to alter the likelihood of response. FSH and LH levels fell in eight of nine and in six of nine patients, respectively (table 3).

Eleven patients had previously received endocrine therapy and two patients who responded had previously responded to tamoxifen therapy. Two of the nine nonresponders had responded to aminoglutethimide and two had responded to androgens.

Side effects were mild in all patients (lethargy [four patients], peripheral edema [three], and hot flushes [two]) and decreased after dose reduction. Side effects did not necessitate withdrawal of therapy in any patient, but two patients required thiazide diuretics.

DISCUSSION

This preliminary study indicates that danazol may be effective treatment for some patients with metastatic breast cancer. Seven of 41 (17%) patients responded to the treatment which was well-tolerated in the doses administered. Although this study indicates that responses occur more frequently in soft tissue sites, bone metastases may well show evidence of healing after a longer followup period and thus no preferential site of action can be inferred at this stage. Similarly, the small number of premenopausal patients treated precludes any statement concerning its efficacy in this group. Studies are presently being carried out to determine whether a higher dose of danazol will be more effective in these patients, since menses were not suppressed in two patients.

The lack of undesirable side effects such as those seen with estrogens and androgens (9) may make danazol an alternative therapy for postmenopausal advanced breast cancer. Side effects were observed mainly at the higher dose of 600 mg/day and they rarely persisted when the dose was reduced to 300 mg/day. Excessive fluid retention occurred at this low dose in two elderly patients necessitating administration of a thiazide diuretic; this permitted danazol therapy to be continued.

The mode of action of danazol is still unclear. It is reported to have antiprogestational (10,11) and androgenic activities in addition to the observed effect in reducing gonadotropin secretion. It may therefore act directly on the tumor, since cytoplasmic progesterone receptor is present in a proportion of breast carcinomas (12). Addition of danazol to an antiestrogen such as tamoxifen may increase the response rate and/or duration of response of breast cancer to these agents. Studies to test this possibility are now underway, both in animal models and human breast cancer.

TABLE 3.—Changes in FSH and LH following danazol therapy

Patient No.	Age (yrs)	FSH (μg/liter) Before danazol	FSH (μg/liter) After danazol	LH (μg/liter) Before danazol	LH (μg/liter) After danazol	Response*
1	74	30	20	12.8	4.3	PR
2	50	> 50	50	24	21	PD
3	60	> 50	3.4	10.9	< 1	PD
4	58	50	20	20	3.5	PD
5	47	> 50	14	38	1.2	PD
6†	43	9.5	8	5.5	6.8	PD
7	53	50	1.2	1.2	1.2	PD
8	66	50	10	16	1.0	PD
9	60	50	30	12.6	8.1	PD
Means:	57	39.9‡	14.9‡	15.7	5.3	
No. of patients with decreased levels:		8		6		

*PR = partial response; PD = progressive disease.
†Premenopausal patients.
‡Excluding patient Nos. 2, 3, and 5.

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cis-Diamminedichloroplatinum(II) Alone and in Combination in the Treatment of Testicular and Other Malignancies[1,2]

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SUMMARY

A total of 124 patients with malignancy were treated with *cis*-diamminedichloroplatinum(II) (CDDP) alone (58 patients, 121 courses) or in combination with other drugs (66 patients, 176 courses). Toxic effects of single-agent therapy were primarily nausea and vomiting; mild renal hematopoietic, and auditory toxic effects occasionally occurred. Positive responses were seen in ovarian and testicular tumors. Rare responses were also seen in melanoma and bladder carcinoma. CDDP combined with vinblastine and bleomycin resulted in a 39% complete response rate in testicular germ cell tumors. All complete responders and ten patients treated in an adjuvant fashion remain alive and disease-free. Failures to achieve a complete response were associated with bulky extrapulmonary disease, pulmonary disease > 2.5 cm in diameter, and past failure to respond to vinblastine and bleomycin. CDDP combination regimens for acute leukemia and for melanoma were of little value. Combination chemotherapy for head and neck cancer showed activity, including a complete response in one of two patients with nasopharyngeal lymphoepithelioma.

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cis-Diamminedichloroplatinum(II) (CDDP) is a chemotherapeutic agent of proven value in the treatment of testicular malignancy. It also has significant activity against a variety of other tumors, particularly ovarian carcinomas, bladder carcinomas, and epidermoid carcinomas of the head and neck region. From August 1976 to December 1978, we used CDDP alone and in combination with other chemotherapeutic agents in phase II and III trials. This report presents our experience with that agent in 124 patients.

MATERIALS AND METHODS

Fifty-eight patients received 121 courses of CDDP given as a single agent for the treatment of various tumors (*see* table 1). Patients received CDDP iv at a dose of 20 mg/m² daily × 4 every 3–4 weeks. CDDP was administered in 2 liters of 5% dextrose in ⅓ normal saline plus 37.5 g of mannitol over 6–8 hours. Furosemide (40 mg) was administered prior to CDDP therapy. Prehydration was not routinely done. Patients were evaluated by audiogram and creatinine clearance prior to each course of therapy. They were considered ineligible for CDDP therapy

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if audiometry revealed significant bilateral hearing deficits in the 1000–4000-CPS range or if creatinine clearance was < 65 ml/minute. During therapy, serum creatinine was measured daily. CDDP was stopped if the creatinine was increased by 50% or to > 1.5 mg/dl. Patients were premedicated for nausea with either procarbazine or, in a double-blind protocol, oral tetrahydrocannabinol.

Sixty-six patients received 176 courses of CDDP in combination with other agents. Thirty-four patients with testicular germ cell tumors were treated with CDDP, vinblastine, and bleomycin in a regimen modified from that of Einhorn and Donohue (1). These tumors were classed by Dixon and Moore criteria as group II (embryonal carcinoma with or without seminoma; 19 patients), group III (teratoma with or without seminoma; one patient), group IV (teratoma with embryonal carcinoma and/or choriocarcinoma and with or without seminoma; 13 patients), and group V (choriocarcinoma with or without embryonal carcinoma and/or seminoma; one patient). CDDP was given at a dose of 20 mg/m²/day for the first 4 or 5 days of each cycle. Diuresis and hydration were as noted above. Vinblastine was given iv at a dose of 0.15 mg/kg on Days 1 and 8. Bleomycin was given im at a dose of 30 mg on Days 1,8, and 15. Courses were repeated at 3–4-week intervals. Patients whose disease did not progress during therapy were given at least three courses of this regimen. Five additional patients with other tumors (two with mediastinal germ cell tumors, one with a hepatic endodermal sinus tumor, one (male) with a retroperitoneal anaplastic tumor, and one with bladder carcinoma) received the same regimen.

Ten patients with tumors of the head and neck region (eight with epidermoid carcinomas and two with nasopharyngeal lymphoepitheliomas) were treated with a 4-day course of CDDP (20 mg/m²/day, Days 1–4) plus vinblastine and methotrexate (0.1 mg/kg and 40 mg/m², respectively, iv on Days 1 and 8) and bleomycin (15 mg im on Days 21 and 28) (28-day cycles).

Six patients with acute nonlymphocytic leukemia resistant to conventional therapy were treated for 4 days with CDDP as above plus 5-azacytidine (150 mg/m²/day as a continuous infusion for 5 days), vincristine (1.5 mg iv, Day 1), and prednisone (60 mg/day orally for 5 days).

Eleven patients with melanoma were treated with CDDP (20 mg/m²/day for 4 days) plus DTIC (200 mg/m²/day iv for 5 days).

For solid tumors, complete response (CR) was defined as the disappearance of all malignant disease lasting a minimum of 4 weeks, and partial response (PR) was defined as a 50% decrease in the sum of the products of the diameters of measurable tumor lesions, without the appearance of new lesions, lasting a minimum of 4 weeks. Disease progression was defined as the appearance of new lesions or the increase in size of old lesions; all other responses were considered stable. For acute leukemia, CR was defined as the disappearance of all blast cells and a restoration of normal hematopoiesis. PR was defined as a ≥ 50% decrease in marrow blast cell content and the disappearance of circulating blast cells, along with a partial restoration of normal hematopoiesis. Disease progression was defined as a failure to achieve a CR or PR.

RESULTS

CDDP as a Single Agent

Toxicity.—Nausea and vomiting occurred in 67% of patients and were judged to be moderate or severe in 43%. Serious nephrotoxicity was not seen. During therapy, three patients had a transient increase in creatinine during therapy to ≥ 2 mg/dl. Three patients had a fall in creatinine clearance to < 50 ml/minute which excluded or delayed a subsequent course of CDDP. Eight of the 121 courses of CDDP resulted in a wbc count of < 3500 but > 2000/mm³; two courses resulted in a wbc count of ≤ 2000/mm³. A platelet count of ≤ 100,000 but > 50,000/mm³ was seen in four courses; a platelet count of ≤ 50,000/mm³ was seen once. Modest hypomagnesemia was frequent (< 1.5 mg/100 ml, ≥ 1.0 mg/100 ml in 49% of courses; < 1 mg/100 ml in 12% of courses). Hypokalemia also occurred frequently but varied with potassium replacement. One patient developed a mild peripheral neuropathy. Two patients developed transient tinnitus, and six patients had a hearing loss of ≥ 30 decibels (but not clinically apparent).

Response.—Positive responses are shown in table 1. They were seen in two of 12 patients with melanoma (PRs lasting 3 and 6 months), in one of eight with bladder carcinoma (PR lasting 3 months), in two of seven with ovarian carcinoma (one PR lasting 3 months and one CR lasting 25 months), and in two of five patients with testicular tumors (one PR lasting 2 months and one CR lasting 6 months). No positive responses were seen in patients with other diseases.

CDDP in Combination

Thirty-four patients received CDDP, vinblastine, and bleomycin for testicular germ cell tumors. Thirty-three of those patients completed at least one course of therapy and were evaluable for re-

TABLE 1.—Diagnosis of tumors in patients treated with single-agent CDDP

Diagnosis	No. of patients	No. of responders
Testicular germ cell tumor	5	2 (1 CR)
Ovarian carcinoma	7	2 (1 CR)
Renal cell carcinoma	2	0
Bladder carcinoma	8	1
Hodgkin's disease	1	0
Lung carcinoma (small cell)	2	0
Prostate carcinoma	1	0
Osteogenic sarcoma	7	0
Other sarcomas	8	0
Melanoma	12	2
Head and neck carcinoma (epidermoid)	4	0
Thyroid carcinoma	1	0
Totals	58	7

TABLE 2.—Patients with testicular germ cell tumors, who were disease-free when treated with adjuvant CDDP, vinblastine, and bleomycin

Patient No.	Stage* at diagnosis	Prior therapy
1	C	Surgery and chemotherapy
2	B_1	Surgery and chemotherapy
3	B_2	Surgery and chemotherapy
4	C	Surgery and chemotherapy
5	B_3	Surgery and chemotherapy
6	B_2	Surgery alone
7	C	Surgery and chemotherapy
8	B_2	Surgery and chemotherapy
9	C	Surgery and chemotherapy
10	B_2	Surgery and chemotherapy

*From ref 2: B_1 = minimal retroperitoneal node disease (< 6 positive nodes); B_2 = moderate retroperitoneal node disease (≥ 6 positive nodes); B_3 = massive retroperitoneal node disease without extension above the diaphragm or to viscera; and C = disease above the diaphragm or metastatic to viscera.

sponse. One patient died of respiratory failure on Day 1 of the regimen. That patient had severe pulmonary involvement with tumor and had a presumed septic event and rapid respiratory decompensation during this CDDP infusion. Bronchospasm, rash, and hepatic and renal dysfunctions were not noted. Autopsy showed mild pulmonary edema and pneumonia. Death due to drug toxicity cannot be excluded.

Ten of the 33 evaluable patients were treated in an adjuvant setting, having been rendered disease-free by prior surgery with or without chemotherapy. Table 2 shows the initial stage (2) and past treatment of these patients. All ten patients received three courses of CDDP, vinblastine, and bleomycin; six of the ten received up to 1 year of subsequent maintenance chemotherapy (four patients with actinomycin D and two with vinblastine). All ten patients remain alive and disease-free, with a followup ranging from 34+ to 116+ weeks (median, 66+).

Twenty-three patients were treated for evaluable disease. Table 3 summarizes characteristics and responses of these patients. Nine of the 23 patients (39%) achieved a CR with chemotherapy. All patients remain alive and disease-free from 33+ to 153+ weeks after starting therapy (median, 96+ weeks). Six of these ten patients received up to 1 year of maintenance therapy (five with vinblastine alone or in combination and one with actinomycin D).

Four patients (17%) achieved a CR after post-chemotherapy surgical resection of histologically confirmed residual tumor. Three of these four patients had achieved a PR with chemotherapy; one had had stable disease. Two of the four patients remain alive and disease-free at 42+ and 53+ weeks; the others relapsed at 21 and 33 weeks and are alive with disease at 57 and 33 weeks.

Six patients (26%) achieved a PR and were not considered candidates for surgical attempts to resect residual disease. All such patients developed

TABLE 3.—CDDP, vinblastine, and bleomycin in the treatment of patients with evaluable testicular germ cell tumors

Patient No., by response	Dixon-Moore group	Sites of metastases	Extra-pulmonary bulk disease	Prior chemotherapy experience
CR				
1	II	Lung, lymph nodes, liver, bone	No	Yes*
2	II	Lung, lymph nodes	No	Yes
3	II	Lung	No	Yes*
4	II	Lung, lymph nodes, liver	No	Yes
5	II	Lung, lymph nodes	No	Yes*
6	IV	Lung, lymph nodes	No	Yes
7	IV	Lung, pleura, lymph nodes	No	No
8	IV	Lung, lymph nodes	No	Yes*
9	II	Lymph nodes	No	No
PR or stable → surgical CR				
10 (PR)	IV	Lung†, lymph nodes	No	Yes‡
11 (PR)	II	Lung†	No	No
12 (PR)	II	Lung†, lymph nodes	No	Yes*
13 (stable)	II	Lung†, lymph nodes	No	Yes‡
PR				
14	II	Lung, lymph nodes	No	No
15	II	Lung†, lymph nodes, brain	Yes	Yes‡
16	II	Lung†, lymph nodes	No	No
17	IV	Lymph nodes	Yes	Yes*
18	II	Lung†, lymph nodes, liver	Yes	No
19	II	Lung†	No	No
Progressive disease				
20	II	Lung†, bone, liver, skin	No	Yes
21	V	Lung†, brain, bone	No	Yes‡
22	IV	Lung†, lymph nodes, muscle	Yes	No
23	IV	Endotracheal	No	Yes*

*Received but did not fail vinblastine and bleomycin therapy.
†Diameter > 2.5 cm.
‡Failed vinblastine and bleomycin therapy.

progressive disease (range, 8–33 weeks; median, 18 weeks). Two of those patients have died; median survival for the group will exceed 38 weeks. Four patients remain alive: three with disease at 32, 42, and 74 weeks, and one disease-free at 86+ weeks after treatment with "supralethal" therapy followed by autologous marrow rescue.

Four patients (17%) had disease progression following chemotherapy. They died at 4, 6, 14, and 38 weeks.

Factors which suggested a low chance of CR to chemotherapy included bulky extrapulmonary disease (> 8 cm; none of four CRs), past failure after receiving vinblastine and bleomycin therapy (none of four CRs), and a pulmonary tumor > 2.5 cm (none of 11 CRs). No inferences could be drawn about response rate as a function of metastatic site or histology, but it is of interest that the one patient with Dixon-Moore group V disease (choriocarcinoma) failed chemotherapy. Lack of any prior chemotherapy did not predict for a CR, since six of eight such patients failed to achieve a disease-free status with chemotherapy alone. Patients with pulmonary disease ≤ 2.5 cm in diameter achieved a 90% CR rate (nine of ten patients). The CR rate in the 19 patients who had not failed prior vinblastine and bleomycin therapy was 47%.

Two of the three patients who received CDDP, vinblastine, and bleomycin for nontesticular germ cell tumors had a positive response. One patient, a 16-month-old infant with hepatic endodermal sinus tumor, achieved a laparotomy-confirmed CR and is alive without evidence of disease 60+ weeks after starting therapy. The two patients with mediastinal tumor did not fare as well. One patient failed therapy, was lost to followup, and is presumed dead. The other patient had a brief PR (~ 4 weeks) and died 60 weeks after starting therapy.

The patient who received CDDP, vinblastine, and bleomycin for bladder carcinoma failed to respond; the patient with an anaplastic retroperitoneal tumor had a PR to chemotherapy lasting 7 months.

Toxicity of 109 courses of CDDP, vinblastine, and bleomycin included nausea and vomiting (85%; 62%

moderate to severe), leukopenia[5] (19 courses with wbc count of < 3500 but ≥ 2000/mm³; eight courses with wbc count of ≤ 2000/mm³), thrombocytopenia[5] (ten courses with platelet count of < 100,000 but > 50,000/mm³; two courses with platelet count of ≤ 50,000/mm³), and nephrotoxicity (two episodes of creatinine ≥ 2.0 mg/dl). There were three episodes of (nonfatal) fever during granulocytopenia. Myalgias were common. One patient developed sialorrhea.

Two patients developed hyponatremia attributed to the syndrome of inappropriate antidiuretic hormone secretion. In one of these patients this syndrome appeared to be due to CDDP since it recurred with a repeat challenge of vinblastine, bleomycin, and CDDP but did not recur with vinblastine or bleomycin alone. Hypomagnesemia (<1.5 mg/dl) was common (> 1.0 mg/dl in 69% of courses), as was hypokalemia. One patient, as noted above, died during the first CDDP infusion but this death may have been due to sepsis and malignancy rather than CDDP toxicity.

Three of eight patients with epidermoid carcinoma of the head and neck region had PRs to therapy (duration: 1, 3, and 3 months). One of two patients with nasopharyngeal lymphoepithelioma had a CR (duration of positive response, 4 months). Toxic effects included nausea and vomiting (60%) and, in two instances, a reduced creatinine clearance to < 50 mg/ml/minute.

None of the six patients with acute leukemia achieved a PR. Two had a marked decrease in peripheral blast cell count. All patients experienced nausea and vomiting. Hematopoietic toxicity was not evaluable due to pre-existing hematopoietic compromise.

One of 11 patients with melanoma achieved a PR lasting 6 months. Nausea and vomiting occurred in 82% of patients (64% severe). Three patients had leukopenia with wbc counts of < 3500 but > 2000/mm³; one patient had thrombocytopenia with a platelet count of < 100,000/mm³. One patient developed a severe allergic reaction to CDDP associated with bronchospam, hypotension, shock, and subsequent death due to hepatic and renal failure.

DISCUSSION

CDDP given as a single agent was associated with acceptable toxicity. Nausea and vomiting were frequent but generally did not limit acceptance of the agent. Nephrotoxicity, auditory toxicity, hematopoietic toxicity, and electrolyte abnormalities occurred but were not severe. CRs and PRs were seen in patients with ovarian and testicular tumors, but efficacy appeared to be less than that reported with combination regimens utilizing CDDP for both ovarian (3,4) and testicular (1) tumors. Low activity was seen in bladder cancer and melanoma. No responses were seen in 14 patients with sarcomas or in occasional patients with renal cell carcinoma, small cell carcinoma of the lung, prostate carcinoma, or thyroid carcinoma. These results are generally consistent with the experience of others (5–7) and reinforce the importance of CDDP as an agent in the treatment of testicular and ovarian tumors. We observed, however, only marginal utility of that drug in other tumor types. The failure of CDDP in sarcomas is noteworthy, in view of the positive results in osteogenic sarcoma reported by Ochs et al (8). We also noted poorer results than expected with bladder carcinomas.

CDDP, given in combination with other agents was of clear value in the treatment of testicular malignancies. The CR rate of 39% due to chemotherapy alone (47% in patients who had not failed prior vinblastine and bleomycin therapy), however, is less than the 70% reported by Einhorn and Donohue (1) but close to the 51% reported by Samson et al (9) with similar regimens. In this series, failure to achieve a CR was more likely in patients with bulky extrapulmonary disease, in patients with a pulmonary tumor > 2.5 cm in diameter, and in patients who had failed prior vinblastine and bleomycin therapy. Einhorn and Donohue (1) noted CRs in six of nine patients with advanced pulmonary disease and in eight of 16 patients with advanced abdominal and pulmonary disease; they excluded from analysis three patients with "massive tumor" who died within 2 weeks of institution of therapy.

In this series most patients who failed to achieve a CR had residual disease too extensive for resection, but two of four patients who achieved a surgical CR are currently alive and disease-free.

While our sample size is small and our regimen is less intensive than that of Einhorn and Donohue (1), one must consider the possibility that, in poor risk patients, the ultimate efficacy of combination chemotherapy for this disease with CDDP, vinblastine, and bleomycin may be less than that suggested by early studies.

All patients given adjuvant chemotherapy are currently alive and disease-free. Einhorn and Donohue (10) have suggested that adjuvant therapy is not necessary because of the ability to recapture and presumably cure with chemotherapy recurrent disease in patients initially treated with surgery alone. Our results support this premise for patients whose recurrent disease is diagnosed early, but sug-

[5]Measured during therapy and on Day 22 or Day 29, but not at the expected nadir.

gest that patients whose disease is detected at a late stage may fail to be cured by combination chemotherapy. While close post-surgical followup may allow early diagnosis of relapse in many patients, it is possible that selected groups of patients (eg, patients with extensive tumor prior to resection, patients whose tumors do not produce β-human chorionic gonadotropin or α-fetoprotein, and patients who are not reliable as to followup care) might fare better with adjuvant chemotherapy than with a watchful waiting approach. It is likely that a large controlled trial of adjuvant therapy will be necessary to resolve these issues.

Our experience with nontesticular germ cell tumors (mediastinal and hepatic) is small but reflects the activity of CDDP, vinblastine, and bleomycin in that setting and supports other data suggesting that patients with mediastinal germ cell tumors are less likely than those with testicular tumors to achieve a CR (11).

The regimen of CDDP, vinblastine, and bleomycin was generally well-tolerated, but one death possibly related to drug toxicity did occur.

In other tumors, CDDP-based combination regimens were not notably effective. Results in the treatment of melanoma have been reported in more detail (12) and suggest that DTIC plus CDDP is no better than DTIC alone. The efficacy of the combination regimen used for epidermoid tumors of the head and neck is not clearly different than that expected with methotrexate alone and is less than that reported by others with similar regimens (13,14). It is of note, however, that one of the two patients with nasopharyngeal lymphoepithelioma had a clinical CR. The combination regimen used for acute leukemia was not of clear value.

Toxicity of the CDDP combination regimens was generally acceptable, except for one patient with melanoma who died after a presumed allergic reaction to CDDP.

CDDP remains an important part of combination chemotherapy in the treatment of germ cell tumors, testicular and other. Treatment of such tumors with combinations of CDDP, vinblastine, and bleomycin has resulted in high CR and CR plus PR rates as well as prolonged disease-free survival for complete responders. Poor-risk patients, with a low CR rate, can be identified. This regimen appears to be effective in the adjuvant setting. It is not yet clear which, if any, patients should receive adjuvant therapy.

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Nephrotoxicity of *cis*-Diamminedichloroplatinum(II) as Measured by Urinary β-Glucuronidase[1,2,3]

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SUMMARY

Urinary β-glucuronidase activity as a measure of renal tubular damage was studied in 23 patients receiving 43 courses of *cis*-diamminedichloroplatinum(II) therapy. A 250% increase in urinary β-glucuronidase activity was noted after therapy, compared with values before therapy. There were no patients who sustained renal insufficiency as evidenced by more commonly used and coarser parameters of renal function. This marked rise in urinary β-glucuronidase activity was felt to indicate tubular toxicity due to the *cis*-diamminedichloroplatinum(II) not measured by the usual parameters of renal function.

[Cancer Treat Rep 64:1083–1086, 1980]

Urinary β-glucuronidase activity (UBGA) has been noted to be elevated in diseases with renal tubular disruption (1–3). Its use has been suggested as a marker for tubular damage by nephrotoxic agents (4).

This study was designed to investigate the renal damage in patients with malignant lesions receiving protocol therapy with the antitumor agent *cis*-diamminedichloroplatinum(II) (CDDP), using UBGA as an indicator of such damage. UBGA was compared with other more commonly used parameters of renal function, such as serum creatinine, BUN, creatinine clearance, urine protein excretion, and routine urinalysis. Its usefulness in terms of sensitivity in monitoring patients for nephrotoxicity due to the CDDP is discussed.

MATERIALS AND METHODS

UBGA was determined by the phenolphthalein glucuronic acid assay as outlined by Leonard (5). Urine (0.2 ml), acetate buffer (0.1 ml of 0.5 M; pH 4.9), phenolphthalein glucuronic acid (Sigma) (0.2 ml of 0.01 M), and distilled H_2O (0.4 ml) were incubated for 4 hrs at 37°C. Three milliliters of glycine buffer (pH 10.5) (6) were added to stop the reaction and to develop the phenolphthalein color. Using a Bausch and Lomb Spectronic 20 colorimeter at 550 nM, the percent transmission of each sample was determined and compared to a standard phenolphthalein curve. To correct for urine flow, micrograms of phenolphthalein liberated/0.3 ml of urine were factored by the urine osmolality. Assuming 1000 g of water equals 1000 ml, this term is then

$$\frac{\mu g \text{ phenolphthalein}/0.3 \text{ ml}}{\text{mOsm}/1000 \text{ ml}},$$

or when this is multiplied by 10^3, the units become

$$\frac{\mu g \text{ phenolphthalein}}{0.3 \text{ mOsm of solute}}.$$

Using this method, UBGA was determined for our laboratory with voided urine specimens from

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125 healthy volunteers (males and females, 18–50 years old) who were receiving no medication. It was previously determined that no appreciable change in enzyme activity due to the presence of inhibitors or activators occurs with short periods of refrigeration (7). Because the urine specimens were rapidly refrigerated, it was not necessary to dialyze the specimens to remove inhibitors or activators.

In a patient population, the urinary enzyme was first studied in groups who had either nonoliguric acute renal failure or oliguric acute tubular necrosis but who had not received CDDP. This was done to determine the accuracy of the method. The criteria considered in making these diagnoses included clinical course, routine urinalysis, urine sodium and osmolality, fractional excretion of sodium, and urine-to-plasma urea and creatinine ratios. Two patients with presumed oliguric acute tubular necrosis had renal biopsies demonstrating the typical anatomic changes of acute tubular necrosis (8). UBGA was measured as part of these patients' initial workup.

UBGA was then studied in cancer patients receiving CDDP and no other known nephrotoxic agents. The chemotherapy protocol included CDDP administered iv at a dose of 50 mg/m^2 over 4 hrs in 500 ml of 0.9% saline with 40 mg of furosemide and 37.5 g of mannitol on 2 successive days. Immediately prior to this infusion, the patients received iv hydration with 1000 ml of 5% dextrose and 0.45% saline over 4 hrs. Following the CDDP, mannitol, and furosemide infusion, the hydration was continued with 5% dextrose and 0.45% saline at the rate of 125 ml/hr for 8 hrs. UBGA was measured before the initial saline infusion and again 12–16 hrs after the discontinuation of the Day-2 course of CDDP. Other tests of renal function before and up to 2 weeks after the course of CDDP included routine urinalysis, urine protein excretion, creatinine clearance, BUN, and serum creatinine.

To ensure that the mannitol and furosemide included in the CDDP infusion were not responsible for increased UBGA after therapy, four normal volunteers received the mannitol and furosemide without CDDP. UBGA was measured before and 16 hrs after infusion.

To ensure that a false elevation in the UBGA, as determined by the phenolphthalein glucuronic acid assay, could not be caused by excretion of CDDP in the urine, CDDP was added to the urine of five normal volunteers at a concentration of 10 μg/ml. A significant portion of CDDP is excreted unchanged in the urine soon after administration. This concentration added to normal urine represents an average or high urinary excretion level which might occur 12–18 hrs after an infusion of CDDP (9). UBGA was measured before and after the addition of CDDP.

TABLE 1.—UBGA activity in urine specimens from normal subjects over varying ranges of concentration*

Urine osmolality (mOsm/kg of H_2O)	No. of subjects	Mean UBGA activity (± SEM) as μg of phenolphthalein/0.3 mOsm of solute
≤250	21	10.4 ± 1.8
251–500	26	9.3 ± 1.1
501–750	26	7.9 ± 0.7
751–1000	26	7.9 ± 0.9
>1000	26	9.7 + 1.0
Total	125	9.0 + 0.5

*All differences between these groups were insignificant ($P > 0.1$).

RESULTS

The normal level of UBGA determined in this laboratory for 125 normal volunteers receiving no medication was < 20 μg of phenolphthalein liberated/0.3 mOsm of solute. This value encompassed 96% of normal subjects in this series (table 1).

Five patients diagnosed as having nonoliguric acute renal failure had increased UBGA (mean ± SEM, 39.2 ± 5.9 μg). The highest level noted was 63 μg and the lowest level noted was 23 μg. Markedly increased UBGA was found in 14 patients with oliguric acute tubular necrosis (281 ± 49 μg). The highest level was 718 μg and the lowest level was 58 μg. These differences were statistically significant ($P < 0.01$).

In 23 patients, UBGA measured during 43 courses of CDDP therapy revealed a mean pretreatment level of 16.8 ± 1.4 μg which increased to 58.4 ± 6.4 μg after therapy ($P < 0.001$). Saline, furosemide, and mannitol infusion without CDDP resulted in a slight fall in mean UBGA (13.8 ± 1.4 to 12.1 ± 1.0 μg) ($P > 0.3$). The addition of CDDP to the urine resulted in no change in UBGA: the mean levels before and after addition of CDDP were identical (9.7 ± 1.9 μg) ($P > 0.3$) (fig 1).

The serum creatinine of 1.0 ± 0.01 mg/dl (mean ± SD) and the BUN of 14.6 ± 4.1 mg/dl (mean ± SD) before CDDP were statistically the same as the serum creatinine of 1.0 ± 0.08 mg/dl and the BUN of 15.8 ± 4.2 mg/dl after CDDP therapy; protein excretions were also the same. The creatinine clearance which by definition cannot change without change in the serum creatinine were also identical. Occasional renal tubular cells and granular casts were noted in the urinalysis before and after CDDP therapy.

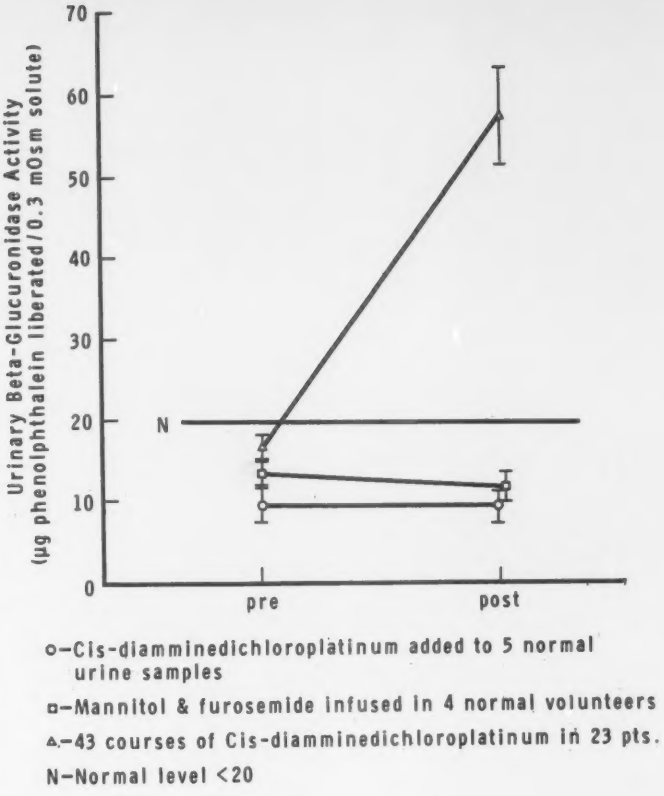

FIGURE 1.—UBGA activity before (pre) and after (post) treatment (mean ± SEM).

DISCUSSION

β-Glucuronidase is a cellular hydrolase found in the urine which is of such sufficiently high molecular weight (218,000) that none is filtered by the normal glomerulus (1). Urinary β-glucuronidase is essentially the product of renal tubular and uro-epithelial cell contribution in the absence of massive proteinuria (7). UBGA is not affected by pyuria and bacteriuria (10); however, Bank and Bailine (1) found urinary levels of the enzyme increased in patients with active renal parenchymal infection. Levels are also increased in acute tubular necrosis (2). The initial portion of this investigation showed modest elevation of UBGA in patients with nonoliguric acute renal failure, while the massive elevations previously noted in patients with oliguric acute tubular necrosis were confirmed.

CDDP is an antitumor agent which has been under clinical trial since 1972 and has been shown to be effective in testicular, ovarian, bladder, and head and neck tumors (11), although its clinical usefulness is limited by its dose-dependent nephrotoxicity. Phase II studies have demonstrated a 25% incidence of acute renal insufficiency in some series (12). The incidence rises from 25% at a dose of 50 mg/m² to 100% at a dose of 200 mg/m² (12–14). The course of nephrotoxicity is characterized by elevation of the serum creatinine which peaks at 3–14 days (14,15). Transient proteinuria and hyperuricemia (16) have also been noted, usually with full functional recovery. Renal functional impairment may be irreversible, especially with the use of high doses and repeated courses, or in patients with preexisting renal disease (16,17). The main pathologic findings are the typical changes of nephrotoxic acute tubular necrosis (9,15,16,18,19).

More recent studies indicate that damage to renal function is preventable by a slower infusion of

CDDP, adequate hydration, and concomitant therapy with mannitol and/or furosemide (11). Patients in the present study received all of these precautionary measures. As noted, no signs of nephrotoxicity, as evidenced by an increase in the BUN and serum creatinine, a decrease in creatinine clearance, or an increase in protein excretion, were found, even with repeated CDDP treatment. However, renal functional damage was demonstrated by the marked increase in UBGA after therapy. There was a $< 25\%$ increase in only two patients. The possibility of a false increase in UBGA due to the excretion of CDDP in the urine and its chemical effects on the method was eliminated. The possibility of renal toxicity due to mannitol and furosemide was also excluded by the overall decrease in pre- and post-infusion levels (14%) in the normal subjects receiving these agents without CDDP. Since CDDP is known to be highly nephrotoxic when given alone and furosemide and mannitol are known to be protective, it is unlikely that the combination of these three drugs is necessary to cause the increase found in UBGA. Since the highest levels of β-glucuronidase in the kidneys are found in the tubular cells, especially the proximal tubular cells (20), the release of β-glucuronidase into the urine is noted in diseases causing damage to the tubular cells. CDDP, having been shown to be such a tubular toxin, is felt to be the single offending agent causing the marked increase in UBGA.

In summary, nephrotoxicity is universally present with CDDP therapy when measured by the more sensitive UBGA assay. Severity of the nephrotoxicity as measured by BUN, creatinine, urine protein excretion, urinalysis, and creatinine clearance appears to be markedly decreased by the slow iv infusion of the drug with adequate hydration and concomitant mannitol and furosemide therapy. The significance of this increased UBGA with regard to long-term and cumulative nephrotoxicity needs to be established.

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High-Dose Metronidazole: Pharmacokinetics and Bioavailability Using an Iv Preparation and Application of Its Use as a Radiosensitizer[1,2,3]

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SUMMARY

As an extension of studies on the clinical use of nitroimidazoles as radiosensitizers, single-dose pharmacokinetic studies of iv metronidazole (500 mg/100-ml vials) were performed in eight consenting patients. Single doses of 0.5, 1.0, 1.5, and 2.0 g (0.29–1.21 g/m^2) were administered iv by a zero-order infusion pump. Serial timed blood and urine samples were assayed for metronidazole and its two metabolites (acetic acid and ethoxy compounds) using a high-pressure liquid chromatographic assay. Open two-compartment kinetic characteristics of metronidazole were computed from simultaneous plasma infusion and urine excretion-rate equations using a nonlinear least-squares regression analysis program (NONLIN). Means of the four kinetic parameters were (h^{-1}): k_{12}, 1.18; k_{21}, 0.86; k_{10}, 0.22; and k'_e, 0.46 × 10^{-4}. Means of the apparent volumes of distribution were (liters/kg): V_c, 0.41; V_B, 1.02; and V_{ss}, 0.75. The mean (± SD) for α-half-life was 1.2 ± 1.3 hours, and that for β-half-life was 9.8 ± 5.9 hours. Seven of the eight patients received a second identical dose orally 1 week later, and the absolute bioavailability was estimated to approximate 100%. Unless the oral route is not feasible and if immediate high peak blood levels are not necessary, oral metronidazole is the preferred route of administration of metronidazole for its radiosensitizing effects.

[Cancer Treat Rep 64:1087–1095, 1980]

The development of 5- and 2-nitroimidazole compounds (metronidazole and misonidazole) as specific chemical radiosensitizers of hypoxic tumor cells has been reviewed (1,4), and these two drugs have been shown to be active in both in vitro (5) and in vivo (6) systems. Earlier studies reported the use of metronidazole orally at doses of 6 g/m^2 (28), with preliminary pharmacologic reports of blood, cerebrospinal fluid, and tissue levels, pharmacokinetics, acute and chronic toxicity, and normal tissue radiation enhancement effects. Using orally administered metronidazole, peak blood concentrations are reached at 2–4 hours after dosing, depending on the amount of drug ingested. Tissue concentrations lag approximately 2 hours after the maximal blood concentration is achieved, while cerebrospinal fluid concentrations fluctuate almost simultaneously with the blood levels. With the previously reported spectrophotometric assay (28), the biologic half-life of metronidazole has been estimated to be very long (10–14 hours).

The isolation and identification of major oxidative metabolites of metronidazole (25), the descriptions of a high-pressure liquid chromatographic (HPLC)

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assay (12,15,30) which will identify, quantitate, and differentiate the parent drug from its metabolites, and the availability of an iv formulation of metronidazole led to this review of the pharmacokinetics of this drug as they apply to the study of hypoxic cell sensitizers. Experience with iv metronidazole in phase II studies has been reported (8,22,30). The iv administration of metronidazole in these phase I studies allows for accurate kinetic analysis and estimation of absolute bioavailability. High doses were used in this study to determine peak plasma and tissue concentrations necessary for a radiosensitizer effect.

MATERIALS AND METHODS

Materials

Pure metronidazole powder was kindly supplied by Poulenc Canada Ltd for standardization of in vitro assays. The structure of metronidazole is shown in figure 1a. The two water-soluble urinary metabolites of metronidazole as supplied by Rhone-Poulenc, France, were the acetic acid metabolite (1-acetic acid-2-methyl-5-nitroimidazole) (fig 1b) and the ethoxy metabolite (1-2-hydroxy-2-hydroxymethyl-5-nitroimidazole) (fig 1c).

Iv metronidazole for clinical investigational use was supplied by May and Baker (England) and obtained through Poulenc Canada Ltd. It was supplied in 100-ml vials with a concentration of 5 mg/ml. Oral metronidazole was the pharmacy stock of 250-mg tablets distributed by Poulenc Canada Ltd. All other chemicals and supplies were purchased from regular commercial suppliers.

Patients

Eight consenting adult patients (five males and three females) were admitted to the radiotherapy service at the Cross Cancer Institute (table 1); their ages ranged from 23 to 77 years. Five of the patients had localized disease while three had metastatic disease. All patient volunteers were clinically preassessed to have normal liver, renal, and hematologic functions. Informed consent was obtained after the nature of the procedure was fully explained.

Metronidazole was administered iv into an antecubital vein using a zero-order infusion pump. Rates of drug administration varied between patients so that the total volume was delivered in a constant time interval of 30 minutes. Each patient received only a single iv dose of metronidazole, with sequential patients receiving incrementally larger doses from 0.5 to 2.0 g (table 1).

Serial blood (via heparin lock in the opposite antecubital vein) and urine (timed cumulative excretion) samples were obtained for drug assay and subsequent kinetic analysis.

Patient 6, whose metastatic disease involved subcutaneous nodules, volunteered to have three nodules biopsied for assessment of drug distribution in tumor tissue (½, 1, and 4 hours after the single iv metronidazole dose of 1.5 g).

Sample Preparation and HPLC Assay

Heparinized blood samples were collected and centrifuged at 2500 × *g* and the plasma fraction was diluted 1:1 with 10% trichloroacetic acid (TCA). After centrifugation, the supernatant was injected into the high-pressure liquid chromatograph. Urine was also diluted 1:10 with 10% TCA and was cen-

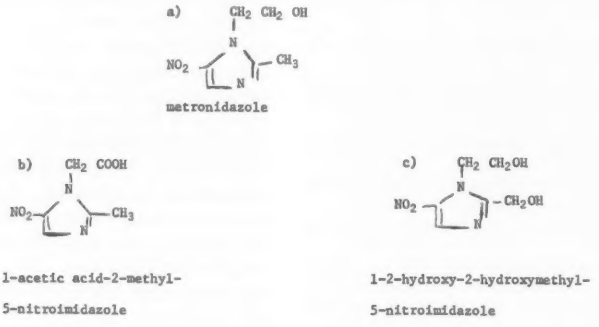

FIGURE 1.—Chemical structure of metronidazole (a) and its two oxidative urinary metabolites: acetic acid metabolite (b) and ethoxy metabolite (c).

TABLE 1.—Characteristics of the 8 patient volunteers and doses of metronidazole administered

Patient No.	Age (yrs)	Sex	Weight (kg)	Dose of metronidazole		Diagnosis
				g	g/m²	
1	52	M	72	0.5	0.29	Metastatic bronchogenic and oat cell carcinomas, lung
2	77	F	53	0.5	0.35	Adenocarcinoma, lung
3	23	F	61	1.0	0.63	Grade II ependymoma
4	29	M	100	1.5	0.71	Grade II astrocytoma
5	66	M	62	1.5	0.88	Bronchogenic carcinoma
6	77	M	55	1.5	0.97	Disseminated adenocarcinoma, primary site unknown
7	57	F	89	2.0	1.02	Metastatic bronchogenic carcinoma
8	64	M	65	2.0	1.21	Bronchogenic and oat cell carcinomas, lung

trifuged prior to injection into the chromatograph. Tumor tissue slices (patient 6) were weighed, minced, homogenized, and then diluted 1:9 (wt/vol) with 10% TCA. This mixture was kept at room temperature for 24 hours before centrifugation.

The high-pressure liquid chromatograph was a Spectrophysics Model 3500B equipped with a Schoeffel Model 770 variable wavelength detector. A Spectrophysics SS-3-250 stainless steel column with reverse-phase Spherisorb ODS-5 micron packing was used with 10% acetonitrile (pH 3.2) solvent at a flow rate of approximately 2.4 ml/minute (pressures of 4000–4500 psi). Samples (10 μl) were injected and peak heights were recorded at a wavelength of 314 nm. Standard curves of metronidazole and its two metabolites (diluted in pooled human plasma) were made with each assay lot, and the peak height:concentration relationship was linear at the drug concentration used in this investigation. No internal standardization was used.

Pharmacokinetic Calculations

Kinetic characteristics of metronidazole were calculated from simultaneous plasma infusion (C_p) versus time and urine excretion rate (dX_u/dt) versus time equations using NONLIN (16), a nonlinear least-squares regression analysis computer program. The equations described are all referenced by Gibaldi and Perrier (11). An open two-compartment model with excretion from the central compartment was used to describe the biexponential decline in C_p or dX_u/dt as a function of time after iv injection. The kinetic parameters D_o/V_c (dose divided by the apparent volume of the central compartment) and k_{10}, k_{12}, k_{21}, and k'_e (the first-order rate constants) were estimated by NONLIN, given the general equation of the model:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \text{ and } dX_u/dt = A'_e{}^{-\alpha t}, B'_e{}^{-\beta t},$$

where A, A′, B, and B′ are the zero-time intercepts of the rapid and slow components of the curve, respectively, and α and β are proportional to the respective slopes of the same two components of the curve. Some of these estimates are used to calculate the following:

(*a*) half-life of the rapid component (h): $t/2_\alpha = 0.639/\alpha$;

(*b*) half-life of the slow component (h): $t/2_\beta = 0.693/\beta$ (terminal phase half-life or biologic half-life);

(*c*) apparent volume of distribution in the body (liters): $V_B = \frac{V_c \cdot k_{10}}{\beta}$;

(*d*) apparent volume of distribution at steady state (liters): $V_{ss} = \frac{V_c(k_{21} + k_{12})}{k_{21}}$;

(*e*) total-body clearance of the drug (ml/minute): $\frac{V_B \cdot \beta}{60} = \frac{V_c \cdot k_{10}}{60}$; and

(*f*) area under the curve (AUC) ($\mu g \cdot h \cdot ml^{-1}$): $AUC = \frac{D_o}{k_{10} \cdot V_c}$.

Bioavailability Calculations

Seven of the eight patients (Nos. 2–8) volunteered to return 1 week later for a repeat kinetic study, this time with a single oral dose of metronidazole identical to their previous iv dose. This dose was administered in 250-mg tablets with a constant volume of water on a 12-hour fasting stomach.

Blood and urine samples were collected and assayed as described above. Kinetic analysis was performed using the NONLIN program according to the general equation for oral absorption into the two-compartment open model:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-k_a t}.$$

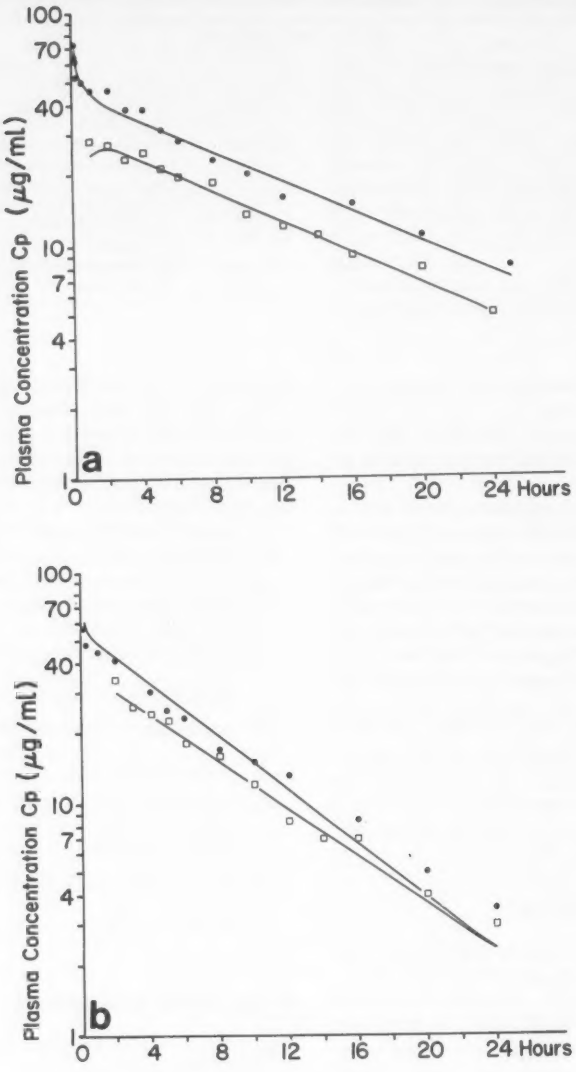

FIGURE 2.—Semilogarithmic plots of plasma concentration (C_p) vs time for: *a*) patient 5 (1.5 g of metronidazole) and *b*) patient 7 (2.0 g of metronidazole). For both patients, iv (●) and oral (□) data are plotted for comparison of AUCs and calculation of absolute bioavailability (63% and 56% for patients 5 and 7, respectively; *see* Materials and Methods section for description of calculation). Points = observed values. Smooth curves = model-predicted concentrations with time, based on computer-derived kinetic constants.

Oral absorption occurred rapidly and therefore evaluation of the absorption-phase kinetics and the estimation of k_a were not possible. Computer-derived data were obtained for graphing purposes (fig 2a and b).

Absolute bioavailability was calculated on the basis of the ratio of AUCs:

$$\% \text{ bioavailability} = \frac{AUC_{(oral)}}{AUC_{(iv)}} \cdot 100.$$

The method for $AUC_{(iv)}$ is described above. The AUC (to infinity) for oral absorption was taken from calculations based on a simple one-compartment analysis:

$$AUC_{(oral)} = \frac{D_o}{V.K},$$

where D_o is the dose administered, V is the volume of the compartment, and K is the elimination rate constant.

RESULTS

Table 2 summarizes the pharmacokinetic parameters (mean ± SD) of distribution and elimination as estimated from the simultaneous plasma infusion versus time and urine excretion rate versus time equations using NONLIN. The rate of decline of plasma drug concentration in the post-distributive phase appears to be independent of dose, and the rate constants appear to be first-order over the range of doses from 0.5 to 2.0 g used in this study. A plot of the mean AUCs at the three doses versus the dose administered is also linear. Therefore, it is assumed that drug concentration in the body at these doses is well below the K_m (Michaelis constant) for metabolism of metronidazole and biotransformation is not rate-limiting. Furthermore, metronidazole is only slightly bound to plasma proteins (8%–20% [26]). It is assumed that metronidazole follows linear pharmacokinetics at these doses.

The data in figure 3 (a and b) are consistent with a linear relationship between dose and peak plasma concentrations. At the end of the 30-minute iv infusion, the peak plasma concentrations were 10, 33, and 71 μg/ml at doses of 0.5, 1.0, and 2.0 g, respectively, in the three patients for whom values are shown. Their biologic half-lives were 6.3, 9.8, and 6.1 hours, respectively. The urine excretion rate data are more variable with time and are included along with the iv data as part of the two-function NONLIN computation.

TABLE 2.—Pharmacokinetic parameters of iv metronidazole distribution and elimination as estimated from simultaneous plasma infusion vs time and urine excretion rate vs time equations using NONLIN

Parameter	Value (mean ± SD)
$k_{12}(h^{-1})$	1.18 ± 1.13
$k_{21}(h^{-1})$	0.86 ± 0.88
$k_{10}(h^{-1})$	0.22 ± 0.10
$k'_e(h^{-1})$	$0.46 \times 10^{-4} \pm 0.25 \times 10^{-4}$
$\alpha(h^{-1})$	2.11 ± 2.07
$\beta(h^{-1})$	0.09 ± 0.04
$t/2_\alpha(h)$	1.24 ± 1.31
$t/2_\beta(h)$	9.76 ± 5.89
V_c(liter/kg)	0.41 ± 0.20
V_B(liter/kg)	1.02 ± 0.44
V_{ss}(liter/kg)	0.82 ± 0.30

*Total-body clearance (ml/min): 106.9 ± 87.4.

Figure 4 shows the timed relationship of plasma and tumor concentrations of metronidazole and its ethoxy metabolite in patient 6. The plasma concentrations peaked at 42 μg/ml after the 30-minute infusion of 1.5 g of metronidazole, and the biologic half-life was 13.6 hours. Tumor concentrations of metronidazole rose to 10.0 μg/ml over the 4-hour sampling interval and would be predicted, based on our previous studies (28), to plateau at this time, although this was not observed. The initial high plasma metronidazole concentrations with the iv administration, in contrast to the 2–4-hour lag time with the oral formulation, did not seem to correlate with earlier peak tissue levels or higher tumor tissue:plasma concentration ratios. As might be predicted pharmacokinetically, the iv formulation offers the advantage of a different route of administration that bypasses any delay due to oral absorption. Tissue levels, however, are still dependent on post-absorptive distribution and the 4-hour lag time is identical to that previously reported with the oral route. The ethoxy metabolite (fig 4) plateaus in 8 hours to low levels of 9–10 μg/ml in plasma and reaches 2.0 μg/ml in 4 hours in tumor tissue. It is of interest to note that metabolites were not detectable in plasma in the three patients given iv doses of 0.5 and 1.0 g. Only low levels (never above 9 or 10 μg/ml) of ethoxy metabolite accumulated at the higher iv doses. With oral administration, low levels and similar plateau concentrations of ethoxy metabolite were detectable.

The percent recovery of metronidazole and its two metabolites (as a percentage of dose administered) was independent of dose and averaged 20%, 30%, and 35.5% at 24, 48, and 72 hours, respectively, after iv administration and 19%, 30%, and 33% at 24, 48, and 72 hours, respectively, after oral administration. The acetic acid metabolite was the

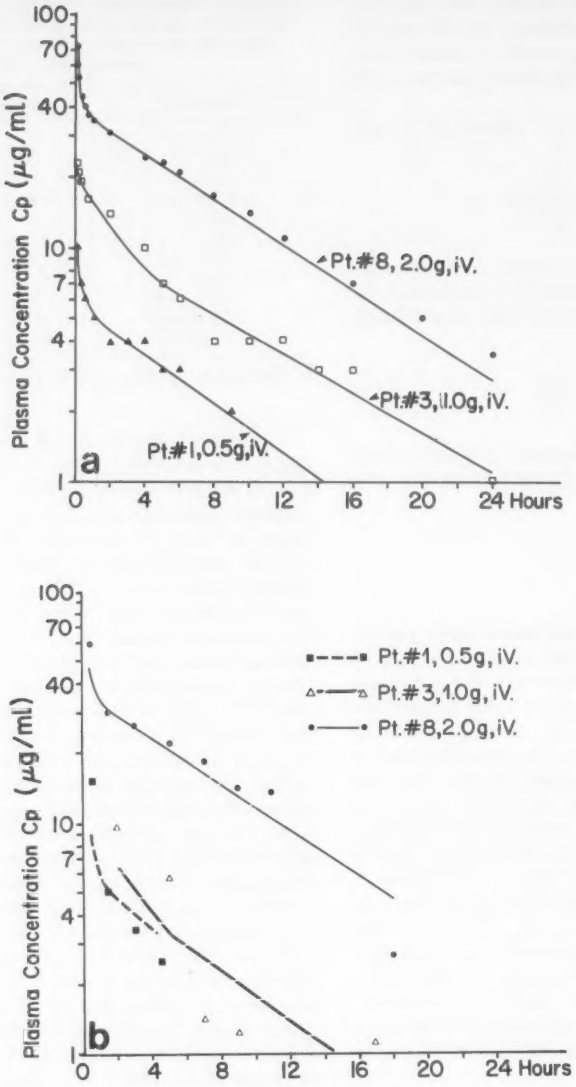

FIGURE 3.—*a*) Semilogarithmic plots of plasma concentration (C_p) vs time for: patient 1 (▲), 0.5 g of metronidazole iv; patient 3 (□), 1.0 g of metronidazole iv; and patient 8 (●), 2.0 g of metronidazole iv. *b*) Semilogarithmic plots of urine excretion rate (dX_u/dt) vs time for same 3 patients. Points = observed values. Smooth curves = model-predicted concentrations with time, based on computer-derived kinetic constants.

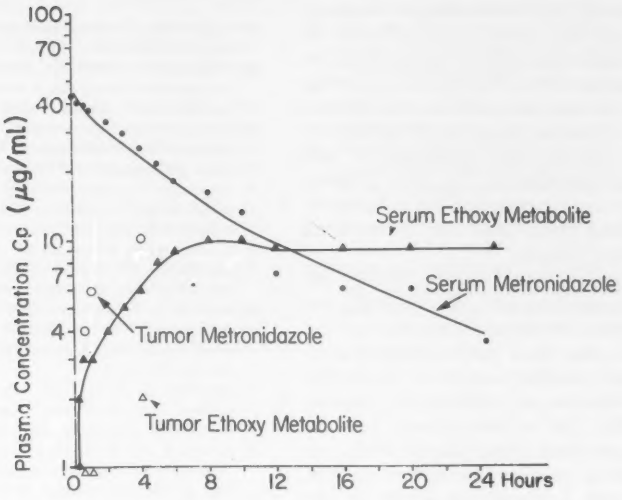

FIGURE 4.—Semilogarithmic plots of plasma concentration (C_p) vs time for patient 6 (1.5 g of iv metronidazole) showing serum metronidazole (●) and serum ethoxy metabolite (▲). Open circles and triangles = skin tumor concentrations of metronidazole and its ethoxy metabolite, respectively, from biopsy specimens taken at ½, 1, and 4 hrs after iv administration of 1.5 g of metronidazole. Points = observed values. Smooth curve = model-predicted concentrations with time, based on computer-derived kinetic constants.

principal compound excreted in the urine in all patients. Cumulative excretion of the acetic acid metabolite, expressed as a percent of total drug excreted (metronidazole plus the two metabolites), was similar after iv and oral administration and averaged 53%, 67%, and 68% at 24, 48, and 72 hours, respectively, after iv administration of metronidazole. Cumulative excretion of metronidazole after iv administration averaged 21%, 19%, and 20% at these same times, whereas cumulative excretion of the acetic acid metabolite after iv administration averaged 26%, 14%, and 12%, respectively.

The absolute bioavailability of oral metronidazole is estimated by calculating the ratios of the AUCs following the oral compared to the iv administration of single identical doses of metronidazole. Three of the seven patients so investigated had ratios > 100%. The mean percent bioavailability ratio in the other four patients was 69% ± 14% (SD), with a range of 56%–89%. Figure 2 (a and b) illustrates the bioavailability in two patients (Nos. 5 and 7) for whom estimates were 63% and 56%, respectively. Accepting the fact that there is considerable variability in estimating AUC, we are led to assume an approximate oral bioavailability approaching 100%.

No gastrointestinal or nervous system toxicity was observed in any of the patients who received the iv preparation.

DISCUSSION

Published assay methods for metronidazole and related nitroimidazoles (eg, misonidazole, ornidazole, and tinidazole) have been reviewed (9) and include a microbiologic bioassay (14,19–21,24), ultraviolet spectrophotometry (27,28), paper and thin-layer chromatography (23,25,29), polarography (3,7,10,13), gas-liquid chromatography and gas-

liquid chromatography plus mass spectrometry (17,31), and HPLC assay (12,15,18,30,32). The ultraviolet assay lacks sensitivity, it is unable to detect metabolites in biologic samples, and urine concentrations are difficult to assay due to background interference. Similarly, the microbiologic bioassays are unable to specifically assay metronidazole as distinct from its metabolites since these metabolites are themselves antibacterial (20). The HPLC assay has the advantage of ease of operation requiring a simple protein precipitation step, no derivatization prior to analysis, reproducible quantitation, and both sensitivity and specificity. The HPLC assay quantitates the two major metabolites of metronidazole, differentiating them from the parent drug in plasma, urine, and tissues.

Using the oral formulation of metronidazole, the pharmacokinetic parameters are not uniformly accurate. The fact that the absorption phase of the kinetic distribution was rapid (and therefore estimation of k_a was not possible) seems to contradict the prolonged distribution and elimination noted in previous studies (28). This is possibly explained on the basis of the large doses administered orally, or possibly on the basis of an enterohepatic circulation of metronidazole with a significant effect on the timed plasma concentration relationship, thus altering the kinetic parameters. The elimination half-life in this study with high-dose iv metronidazole approximates that reported in pharmacokinetic studies using small oral doses (2,26). Plasma levels following high dose iv administration of metronidazole are less influenced by interfering absorption patterns; therefore, a truer distribution and elimination curve results in more accurate kinetic estimates.

In one patient, tumor tissue (total excision of subcutaneous nodules) concentrations after iv metronidazole were shown to lag behind the plasma concentrations in a manner similar to that for oral administration. The immediate high peak plasma levels (as in the central compartment at the end of the iv infusion) do not therefore imply similar high tissue concentrations, although it may be important to have the high peak level in order to increase the diffusion gradient. The disadvantage of the present iv metronidazole preparation is its dilute formulation (5 mg/ml) where a large volume of iv solution is necessary to approximate the usual radiosensitizing doses given orally, requiring a prolonged infusion time of > 30 minutes.

Should present and future nitroimidazole compounds be investigated, a formulation of higher concentration could have the advantage of a shorter infusion time and might therefore result in higher plasma and corresponding tumor tissue levels. In a similar fashion, iv formulations of newer nitroimidazoles would be important if the oral bioavailability of the new compound was appreciably < 80%.

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Effect of Misonidazole Therapy on Human Granulopoietic Stem Cells[1,2]

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SUMMARY

Misonidazole is a 2-nitroimidazole compound currently being assessed as a radiosensitizing agent. The effects of misonidazole on human bone marrow hematopoiesis were assayed by culture of committed granulocyte-macrophage progenitor cells (CFU-C). Three groups of patients with nonhematologic malignancies were selected for study. The first group of patients received a single, large dose of misonidazole; the second group received smaller, multiple doses of misonidazole; and the third group who did not receive any misonidazole served as irradiation controls. In 14 of 16 patients who received single or multiple doses of misonidazole, there was a significant decrease in the number of CFU-C present in the bone marrow after misonidazole therapy. In five patients who received irradiation only, there was no difference in the number of pre- and post-treatment bone marrow CFU-C. In misonidazole treated patients, extensive washing of post-treatment bone marrow samples failed to return CFU-C growth to control values. Suppression of CFU-C growth persisted for 3 weeks and returned to control values by 8 weeks. This reduction in the proliferative capacity of human bone marrow progenitor cells suggests that misonidazole may add to the myelotoxicity already associated with radiotherapy, systemic chemotherapy, or a combination of the two.

[Cancer Treat Rep 64:1097–1102, 1980]

Misonidazole is a 2-nitroimidazole compound currently being used to sensitize hypoxic tumor cells to the effects of irradiation (1,2). Initial in vivo and in vitro studies have indicated that misonidazole has relatively little effect on well-oxygenated cells, but has selective cytotoxic and radiosensitizing effects on hypoxic cells (3,4). It has been suggested that misonidazole be added to systemic chemotherapy protocols in an attempt to make use of its cytotoxic properties toward hypoxic cells (5).[4] To date, the side effects noted with the use of this drug have been nausea, vomiting, hypersensitivity, peripheral neuropathy, convulsions, and ototoxicity (6–22). Because studies have indicated that well-oxygenated tissues are largely spared the cytotoxic and radiosensitizing effects of misonidazole, there has been little concern that the use of misonidazole will enhance the myelotoxicity of irradiation or systemic chemotherapy. However, Lefebvre and Hasseltine (23) and Levrat and co-workers (24) have demonstrated that the use of a similar nitroimidazole compound, metronidazole, results in a reversible neutropenia in a significant number of patients. The observation of this nitroimidazole-associated neutropenia prompted us to determine if misonidazole has any effect on human hematopoiesis.

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[4]Adams GE. Invited lecture to American Society of Therapeutic Radiologists, Los Angeles, CA, 1978.

Studies by Allalunis et al (25) have demonstrated that misonidazole has no cytotoxic or radiosensitizing effect on murine hematopoiesis in vivo as measured by assays of committed granulocyte-macrophage progenitor cells (CFU-C) and pluripotential stem cells. This paper examines the effects of misonidazole on human bone marrow hematopoiesis. In these studies, the hematopoietic status was monitored by the bone marrow CFU-C assay.

PATIENTS AND METHODS

All patients in this study had been previously randomized into approved protocols for the treatment of nonhematologic malignancies. Patients with evidence of hematologic abnormalities or evidence of tumor involvement of the bone marrow were excluded from the study. No patient had received any previous chemotherapy nor was any chemotherapy used during this study. Written informed consent was obtained before each bone marrow aspirate was obtained.

There were three groups of patients (table 1): group A received a single large dose of misonidazole, group B received multiple small doses of misonidazole, and group C served as irradiation controls. In each group, each patient acted as his own experimental control: ie, the value of each patient's pre-treatment bone marrow culture was considered to represent 100% of that patient's proliferative capacity. Subsequently, each patient's post-treatment bone marrow cultures were compared to his own pre-treatment values. Twenty-one patients consented to participate in these studies.

Group A consisted of five patients with bronchogenic oat cell carcinoma. Following a control bone marrow culture, each patient received a single dose of misonidazole (4.7–5 g/m^2, orally). A post-treatment bone marrow culture was obtained from each patient 4 hours after the ingestion of misonidazole and immediately before any radiation therapy. At that time, the plasma concentrations of the drug are at peak levels (17).

Group B consisted of 11 patients with astrocytoma (eight), squamous cell carcinoma (one), meningioma (one), or rectal carcinoma (one). Following a control bone marrow culture, each patient received multiple doses of misonidazole (1.25–2 g/m^2, orally) three times weekly for 3 or 4 weeks. During that time, the patients were treated with fractionated radiation, each fraction administered 4 hours after drug ingestion. Post-treatment bone marrow cultures were obtained after 1, 2, or 3 weeks of therapy. In each case, the post-treatment bone marrow sample was aspirated 4 hours after the administration of that day's misonidazole and immediately prior to that day's irradiation.

Group C consisted of five patients with bronchogenic oat cell carcinoma (four) or astrocytoma (one). Following a control bone marrow culture, three patients with oat cell carcinoma received 800 rads as a single dose of systemic irradiation to the upper half of the body. A post-treatment bone marrow culture was obtained 4–24 hours following irradiation. In the remaining two patients, control bone marrow was sampled before fractionated whole-brain or upper-half body radiation (200 rads/fraction × 5 fractions/week) was administered. A post-treatment bone marrow culture was obtained immediately after the patients had received a total of 1000 rads. In all patients receiving irradiation, bone marrow samples were obtained from sites distant from the field of irradiation. The estimated dose of irradiation scattered to the site of aspiration is 4 rads/fraction.

Misonidazole was kindly supplied in 500-mg capsules by Hoffmann-LaRoche Ltd, Montreal, Canada. Plasma and bone marrow concentrations of misonidazole and its metabolite, Ro-07-9963, were determined by high-performance liquid chromatography (Spectra-Physics Model 3500B).

Bone marrow cells were cultured according to a modification of the techniques described by Pike and Robinson (26). Briefly, 3 ml of heparinized bone marrow was allowed to sediment at room temperature. Leukocyte-rich plasma was removed and duplicate cell counts were obtained with the aid of a Coulter Counter Model ZBI (Coulter Electronics, Hialeah, FL). Cells were added to a mixture of 2% methylcellulose (Fluka) in minimum essential Alpha media (GIBCO) containing 20% fetal calf serum (Flow) at a final concentration of 10^5 cells/ml. One milliliter of this mixture was overlaid onto 10 × 35-mm tissue culture dishes (Corning) containing 10^6 normal human leukocytes suspended in a 0.5% agar (GIBCO) solution. These peripheral leukocytes acted as a source of colony-stimulating activity (CSA). In these experiments, peripheral blood leukocytes were obtained from a single donor. In all cultures, each experimental group contained four to eight replicate plates. All plates are incubated in a humidified atmosphere containing 7.5% CO_2 at

TABLE 1.—Treatment plan

Patient group	Misonidazole	Radiation
A	Single large dose	—
B	Multiple small doses	Yes
C	—	Yes

37°C (National Incubator Co). Colonies of ≥ 50 cells are counted at 12–14 days with the aid of an inverted microscope. In the experiments which required an alternate source of CSA, human mononuclear cells were isolated from peripheral blood by density centrifugation (27) (Ficoll-Hypaque, Pharmacia), and were incubated for 7 days in the presence of Alpha media containing 15% fetal calf serum at a final cell concentration of 2×10^6 cells/ml. After 7 days of incubation, the cells were pelleted by centrifugation. The supernatant was decanted, filtered, and stored frozen.

RESULTS

In all patients studied, the numbers of pre-treatment bone marrow colonies were within normal limits (normal: 12–120 CFU-C/10^5 cells (28); this study: 11–109 CFU-C/10^5 cells).

In four of five patients in group A, a single dose of misonidazole resulted in a reduction in the number of CFU-C present in post-treatment bone marrow samples, when compared to each patient's own control values (table 2). One patient showed an increase when compared to his own control values. In group B, ten of 11 patients receiving multiple doses of misonidazole had a reduction in the number of CFU-C present in their post-treatment bone marrow samples (table 2). One patient showed no change in number. There was no difference in the number of pre- and post-treatment bone marrow CFU-C in any patient who served as an irradiation control (group C) (table 2). In 14 of 16 patients who received single or multiple doses of misonidazole, there was a significant reduction in the number of post-treatment CFU-C when compared to each patient's control value ($P < 0.001$ by paired t test).

In nine patients receiving misonidazole, aliquots of the post-treatment bone marrow samples were washed three times. In each case, a thorough wash failed to restore colony growth to control values (table 3).

When misonidazole was added in vitro to normal human bone marrow cultures at concentrations similar to those found in the bone marrow of treated subjects (100–800 μM), there was no inhibition of colony growth (table 4).

When normal human mononuclear cells were incubated with misonidazole at the upper range of concentrations attained in the plasma of treated patients, there was no change in the ability of the resultant CSA to stimulate normal bone marrow CFU-C growth when compared to that obtained from mononuclear cells incubated without misonidazole.

Four patients were available for followup cultures after the completion of misonidazole therapy. None

TABLE 2.—Comparison of bone marrow cultures before and after misonidazole

Patient group-No.	Total dose (g)	Misonidazole concentration (μM)*		CFU-C/10^5 cells†	
		Plasma	Marrow	Pre-treatment	Post-treatment
A-1	8.0	796	786	12.5 ± 0.6	2.5 ± 0.6
A-2	8.5	746	746	38 ± 2.6	27.8 ± 1.2
A-3	8.0	502	487	34.8 ± 2.3	21.6 ± 0.7
A-4	8.5	186	162	49 ± 1.3	62.5 ± 2.9
A-5	9.5	NA	NA	31.3 ± 2.1	18.5 ± 1.0
B-1	16.5	726	856	29 ± 2.2	4.2 ± 0.7
B-2	21	662	NA	21.6 ± 1.5	0
B-3	6	102	131	14 ± 1.8	8.0 ± 0.4
B-4	7.5	388	458	109 ± 5.1	66.3 ± 4.2
B-5	19	NA	187	14 ± 1.6	6.5 ± 0.6
B-6	7.5	134	112	38 ± 0.9	9.0 ± 0.4
B-7	6.25	333	300	44.5 ± 1.2	2.5 ± 0.4
B-8	7.5	233	199	18.5 ± 0.5	0.8 ± 0.2
B-9	7.5	173	173	22 ± 1.2	22 ± 1.1
B-10	22.5	NA	NA	17.6 ± 0.9	4.6 ± 0.6
B-11	15.0	NA	NA	27.8 ± 1.7	0
C-1	0	—	—	16.5 ± 1.5	15.5 ± 0.5
C-2	0	—	—	37 ± 1.1	34.3 ± 1.2
C-3	0	—	—	11 ± 0.8	13.0 ± 2.0
C-4	0	—	—	16 ± 1	14.8 ± 0.9
C-5	0	—	—	21.3 ± 1.0	21.5 ± 0.5

*At time of post-treatment bone marrow aspiration; NA = not available.
†Mean ± SEM of replicate plates from 1 aspirate.

TABLE 3.—Effect of washing on bone marow culture

Patient group-No.	CFU-C/10^5 cells (mean ± SEM)		
	Pre-treatment	Post-treatment	Post-treatment + wash × 3
A-2	38 ± 2.6	27.8 ± 1.2	24.0 ± 1.4
B-4	109 ± 5.1	66.3 ± 4.2	68 ± 4.5
B-5	14 ± 1.6	6.5 ± 0.6	8.6 ± 1.4
B-6	38 ± 0.9	9.0 ± 0.4	8.0 ± 0.8
B-7	44.5 ± 1.2	2.5 ± 0.4	3.8 ± 0.7
B-8	18.5 ± 0.5	0.8 ± 0.2	0
B-9	22 ± 1.2	22 ± 1.1	23 ± 1.0
B-10	17.6 ± 0.9	4.6 ± 0.6	3.3 ± 0.9
B-11	27.8 ± 1.7	0	0

TABLE 4.—In vitro addition of misonidazole to normal human bone marrow cultures

Misonidazole concentration (μM)*	% control CFU-C/10^5 cells†
0	100
400	93
800	89
1200	84
1500	53
2400	23
3000	0

*Plasma levels (range) attained in treated patients: 112–856 M.

†Based on mean ± SEM of 3 experimental trials.

of these patients had received any form of therapy in the interval following their last dose of misonidazole. Patient B-4 showed a further decline in the number of CFU-C present at 3 weeks. Patients B-6, A-4, and B-5 had CFU-C which returned to control values by 8, 9, and 12 weeks, respectively, following the completion of treatment.

DISCUSSION

These studies suggest that misonidazole is capable of producing a transient depression in the number of bone marrow CFU-C of patients receiving single or multiple doses of this drug. This damage to the CFU-C appears to be reversible since proliferative capacity returned to normal within 8–12 weeks in those patients who had followup cultures.

The suppression of CFU-C growth was independent of irradiation. The single-dose misonidazole group (group A) had pre- and post-treatment bone marrow aspiration within 4 hours and before any irradiation was administered. Therefore, the observed decrease in the number of CFU-C may be attributed to an effect of the misonidazole alone. Ten of 11 patients who received multiple doses of misonidazole had significant suppression of CFU-C activity. Each post-treatment aspiration was done at a site well-removed from the field of irradiation. The scatter radiation to sites distant from the site of bone marrow aspiration is unlikely to have influenced the bone marrow samples. The maximum radiation scatter estimated from treatment planning protocols is < 2%/fraction. Furthermore, in the patients studied who received radiation without misonidazole (group C), the irradiation had no immediate effect on the number of CFU-C present in post-irradiation bone marrow cultures obtained from sites outside the field of irradiation.

Misonidazole per se is unlikely to be the substance responsible for the decline in proliferative capacity. The addition of misonidazole to control cultures of normal human bone marrow in amounts corresponding to peak levels attained in the bone marrow of treated patients did not reduce the proliferation of CFU-C despite the continued presence of high levels of the drug throughout the 14-day incubation period. Only when misonidazole was added at concentrations far exceeding those obtained in vivo was significant inhibition of CFU-C noted (table 4). These results are similar to those observed by Taylor and Rauth (29) in their studies of HeLa and Chinese hamster ovary cells incubated in the presence of various concentrations of misonidazole. The results from chronic toxicity tests indicate a gradual decrease in plating efficiency in the range of 100–1000 μM misonidazole, with a rapid decline in plating efficiency at higher concentrations. The mechanism responsible for cytotoxic damage at high concentrations remains unclear. Investigations with (^{14}C)misonidazole have indicated that following in vitro aerobic incubations, only the unaltered drug or its *O*-demethylation derivative are present in the media (29). However, several metabolites of misonidazole have been detected following in vivo administration (30,31). These findings would suggest that a metabolite of misonidazole which is formed in vivo

is the myelotoxic agent. This metabolite must be rapidly formed since toxicity is evident within 4 hours of ingestion of the drug. The specific metabolite(s) responsible for damage to the CFU-C has yet to be isolated. Multiple washings of the post-treatment bone marrow samples did not abrogate suppression of growth, which also suggests in vivo damage.

Additional experiments attempted to determine if reduced numbers of CFU-C in post-treatment bone marrow samples were the result of an inhibition of granulocyte-macrophage CSA by misonidazole. CFU-C growth requires the presence of CSA. The incubation of CSA-producing mononuclear cells with high concentrations of misonidazole did not interfere with the ability of these cells to manufacture and release CSA, nor was the resultant CSA less effective in promoting growth of normal human bone marrow. It would appear that misonidazole induces damage to the proliferative capacity of the committed stem cells themselves.

In speculating as to the mechanism of misonidazole-induced damage, it may be appropriate to reconsider an assumption made concerning the nature of human bone marrow. It is generally thought that human bone marrow is a uniformly well-oxygenated tissue. However, present techniques do not allow for quantitative measurement to support this assumption. It may be that there are islands of hematopoiesis that are relatively hypoxic to the rest of the bone marrow. Misonidazole may be exerting cytotoxic effects on these progenitors of colony-forming cells. In addition, relatively hypoxic elements of the hematopoietic microenvironment (bone and cartilage) may be producing metabolites of misonidazole which are toxic to both hypoxic and oxic cells. In the in vitro Chinese hamster ovary system, Whitmore et al (32) have postulated that metabolites of misonidazole produced by hypoxic cells are toxic to aerated tissues. Because this evidence is presently being reassessed, its possible application to human bone marrow remains unclear.

These studies have shown that the effect of misonidazole on human bone marrow hematopoiesis are transient and did not result in neutropenia or aplasia in any patient studied. This decline in CFU-C with subsequent return to control values and lack of neutropenia is similar to the pattern of marrow proliferative activity described in patients receiving cytosine arabinoside and 6-thioguanine as maintenance therapy for acute myelogenous leukemia (33). In both instances, the monitoring of CFU-C activity has suggested potential hematopoietic damage without peripheral blood changes.

The myelotoxic effect of irradiation and chemotherapy are well-documented (21). The addition of misonidazole to these regimens may, through additive or synergistic effects, enhance their toxicity, offsetting any potential therapeutic gain.

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Treatment of Metastatic Renal Cancer With Ifosfamide and Mesnum With and Without Irradiation[1]

Sophie Dorothea Fossâ* and Kristian Talle[2]

SUMMARY

Ifosfamide (50–60 mg/kg of body weight, Days 1–5) and mesnum (10–12 mg/kg of body weight, Days 1–5) were given to 15 patients with measurable metastatic renal cancer. This treatment was repeated on Day 29. In addition, six of these 15 patients received irradiation to some of the metastatic areas. There was one partial remission among 11 evaluable patients after two ifosfamide courses. The partial remission was seen in a metastatic area treated with low-dose irradiation prior to the first ifosfamide course. Two cases of early death and two cases of toxic death (urotoxicity) were observed. The main hematologic complication was moderate to severe leukopenia. Previously reported high response rates to ifosfamide treatment of renal cancer could not be confirmed.

[Cancer Treat Rep 64:1103–1108, 1980]

Ifosfamide (Holoxan) is an alkylating cytostatic drug which has shown antitumor activity in various human malignant tumors (1–5). The dose-limiting effects have been myelosuppression and urotoxicity. Urotoxicity is based on the presence of acrolein, which occurs as a metabolic product in the urine of patients treated with ifosfamide (6). Acrolein leads to epithelial injuries in the kidneys and bladder. After mesnum (Mitexan) is added to the ifosfamide treatment, the risk of urotoxicity seems to decrease without reducing the antineoplastic effect of the drug (6–8). Mesnum leads to a nontoxic additive compound with acrolein in patients receiving ifosfamide therapy (6).

Objective response rates of 18%–50% have been reported in patients with metastatic renal carcinoma (1,2,4), which is one of the most drug-resistant cancers. Therefore, a phase II study using ifosfamide and mesnum in patients with measurable and/or evaluable metastatic renal cancer was undertaken to analyze the effect and toxicity of the drug.

MATERIALS AND METHODS

From Dec 1, 1978, to July 1, 1979, 15 patients (ten males and five females) (table 1) with metastatic hypernephroma were treated with ifosfamide. None of the patients had been treated with hormonal or cytostatic agents prior to ifosfamide therapy. Fourteen patients had measurable lung and/or lymph node metastases, and one (patient 10) had evaluable liver and bone metastases. Liver metastases were considered to be evaluable if the liver scan showed unequivocal space-occupying

TABLE 1.—Characteristics of 15 patients treated with ifosfamide and mesnum with or without irradiation

Parameter	Value
No. of patients	15
Mean age (yrs)	55.5 (range, 44–67)
No. of nephrectomized patients	14
Mean interval (mos) between initial diagnosis and 1st metastasis	28.5 (range, 0–115)
Mean interval (mos) between nephrectomy and start of ifosfamide treatment	34.9 (range, 1–118)
No. of patients with previous hormonal and/or cytotoxic treatment	10
Mean Karnofsky index at start of treatment	80.6 (range, 40–100)

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processes. Retroperitoneal tumors were considered to be evaluable if the extent of the tumor was demonstrable by arteriography.

Six patients had simultaneous radiotherapy to some metastases in order to study a possible synergistic effect of ifosfamide and irradiation (1). Four patients (Nos. 8, 9, 11, and 12) received low-dose irradiation (total, 800–1190 rad/4–9 days). Two patients (Nos. 10 and 14) received high-dose irradiation (total, 3000–3300 rad/14–18 days) (table 2). The following dose schedule recommended by Hoefer-Janker et al (1) and Schnitker et al (2) was used: 50–60 mg of ifosfamide/kg of body weight iv daily on Days 1–5. Each injection of ifosfamide was immediately followed by an iv injection of mesnum (10–12 mg/kg of body weight). The mesnum injection was repeated 4 and 8 hours later. Ifosfamide and mesnum were administered through the same veinflow. All patients were heavily hydrated during the ifosfamide treatment. On Days 1–5, 3–5 liters of 0.9% NaCl solution was injected iv. All patients were pre- and post-hydrated with 2 liters of 5% glucose iv. In order to alkalinize the urine, sodium bicarbonate was given orally (1 g three times daily, Days 1–5). The 4-hour urine output was measured from Day 1 to Day 5. The pH value of the urine was controlled six times daily. If the urine output was not satisfactory, 10 mg of furosemide was given iv.

TABLE 2.—Response in 15 patients with metastatic renal cancer treated with ifosfamide and mesnum with or without irradiation

Patient No.	Nonradiated metastases		Radiated metastases			Total evaluation of response
	Localization	Response	Localization	Total irradiation dose/treatment duration	Response	
1	Lung	Disease progression				Disease progression
2	Lung, supraclavicular lymph nodes	Disease progression				Disease progression
3	Lung, supraclavicular lymph nodes	Disease progression				Disease progression
4	Lung	Disease progression				Disease progression
5	Lung, retroperitoneum	Disease progression				Disease progression
6	Lung, liver	Disease progression				Disease progression
7	Lung	Disease progression				Disease progression
8	Lung, subcutis	Disease progression	Lung	200 rad × 4 for 7 days	Disease progression	Disease progression
9			Mediastinum	200 rad × 5 for 8 days	No change	No change
10	Liver, bone	Disease progression	Mediastinum	300 rad × 10 for 18 days	No change	Disease progression
11	Retroperitoneum	No change	Mediastinum	200 rad × 5 for 8 days	Partial response	Partial response
12			Mediastinum, bone	200 rad × 4 for 6 days 170 rad × 7 for 9 days		Toxic death
13	Mediastinum	No change*				Toxic death
14	Supraclavicular lymph nodes	Disease progression*				Early death
15	Lung	Disease progression*	Mediastinum	300 rad × 10 for 14 days		Early death

*Evaluated after only 1 course.

A new course with ifosfamide was started on Day 29. All patients were hospitalized from Day 1 to Day 7.

The tumor response was assessed after at least two ifosfamide courses. As a rule, ifosfamide was discontinued if there was no objective tumor regression after two courses. Complete remission was defined as the disappearance of all symptoms and signs of disease. Partial remission was defined by a decrease of ≥ 50% in the size of the tumor as measured by the product of the two largest perpendicular tumor diameters. In the lesions that could not be accurately measured (eg, mediastinal tumors), the reduction should be at least 75% of the estimated volume. The response of liver metastases was assessed according to Moertel et al (9). No change was reported if no new lesions had appeared, if no existing lesions had increased in size, or if any decrease in size was not sufficient to indicate partial remission. Disease progression was defined as an increase in size by ≥ 25% of the tumor's size or if any new lesions appeared, regardless of what the response of the other lesions may have been. In bone metastases, disease progression was defined as new bone metastases occurring in previously normal areas, demonstrated either by roentgenogram or by unequivocal bone scan. Early death was defined as death due to progressive disease within 3 weeks after the start of treatment without severe drug toxicity. Toxic death was defined as death occurring at any time during the study and due to drug toxicity.

If a patient was treated with both irradiation and ifosfamide the response was evaluated separately in the nonradiated and in the radiated areas (with the exception of patient 9).

During ifosfamide treatment a creatinine clearance was routinely done on Day 1 (before treatment), on Day 3, and on Day 5. Hemoglobin and wbc and platelet counts were measured on Days 1, 3, 5, and 7 and weekly during the treatment-free interval. The urine sediment was examined for rbcs and cylinders on Days 1, 3, and 5 and weekly in some patients during the treatment-free interval.

RESULTS

Only one of the 11 evaluable patients (No. 11; table 2) who received two courses of ifosfamide achieved a partial response of histologically verified lymph node metastases to the mediastinum. The mediastinal tumor was irradiated with a total dose of 200 rad × 5 for 8 days prior to the first course of ifosfamide. In the same patient a nonradiated retroperitoneal tumor remained unchanged. In nine patients the disease progressed after two ifosfamide courses, while in one patient (No. 10) there was no change. In two patients (Nos. 12 and 13) toxic death occurred on Day 10 of the first course and on Day 11 of the second course, respectively. One patient (No. 12) died of uremia. He had severe leukopenia (0.7×10^9 cells/liter) 2 days before he died. The other patient (No. 13) had septicemia after the second ifosfamide course. The day before death many granulated and hyaline cylinders were observed in the urine sediment together with slight microhematuria (3–15 rbcs/view field), and the serum creatinine was moderately increased (261 μmols/liter). An autopsy revealed an acute tubular necrosis in the remaining kidney and a large mediastinal metastatic tumor.

Two additional patients (Nos. 14 and 15) died 3 weeks after the first ifosfamide treatment because of disease progression (early death). Both patients had a large tumor burden when treatment with ifosfamide was started.

Nine patients had moderate leukopenia (≥ 1.0 to < 3.0×10^9 cells/liter), while six patients had severe leukopenia (< 1.0×10^9 cells/liter) during one or both courses (table 3). The nadir wbc count values were seen 8–17 days after the start of ifosfamide treatment (fig 1). Leukopenia lasted from 3 to 10 days. Eight patients had to be treated with antibiotics because of life-threatening infections occurring together with leukopenia. Platelet counts of < 80×10^9/liter were not observed in any of the patients (fig 2). During the first 7 days of the course, the creatinine clearance dropped significantly in all patients, but was usually restored to the initial value 4 weeks after the start of treatment (fig 3).

Five patients had no macrohematuria during treatment with ifosfamide. In five patients a positive BM-test-8 for blood (Boehringer, Mannheim, W Germany) in the urine was observed at least once despite a low rbc count (table 3). Three patients had 3–15 rbcs/view field and two patients had 16–40 rbcs/view field. Hyaline and granulated cylinders in the urine sediment were observed in only one patient (No. 13).

In all patients severe nausea and vomiting were the most troublesome symptoms from Day 1 to Day 5. All patients had total alopecia. In five patients toxic effects from the cerebrum (predominantly hallucinations) were observed on Days 4 and 5 of an ifosfamide course.

After 5 days' treatment with ifosfamide, all patients had pronounced lethargy or fatigue. The Karnofsky index (performance status) was slightly decreased after the second ifosfamide course compared to the initial index. Local thrombophlebitis occurred frequently from Day 3 to Day 5 at the injection site. Three patients refused further treatment after com-

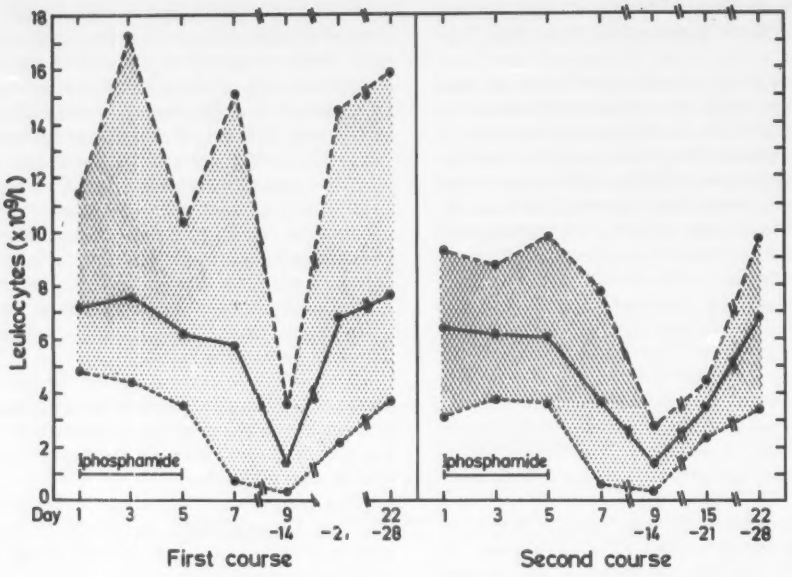

FIGURE 1.—Wbc counts during 2 courses of ifosfamide and mesnum. ———— = mean value, — — — = maximum value, ---- = minimum value.

TABLE 3.—Toxic effects in 15 patients with metastatic renal cancer treated with ifosfamide and mesnum with or without irradiation

Patient No.	Degree of leukopenia*	Life-threatening infection	Cerebral toxic effects	Degree of hematuria†
1	Moderate	—	—	+ +
2	Moderate	Severe	—	(+)
3	Severe	Severe	Hallucinations	+ +
4	Moderate	—	—	0
5	Severe	Severe	Hallucinations	+
6	Severe	—	—	0
7	Moderate	—	—	(+)
8	Moderate	—	—	+
9	Moderate	Severe	—	0
10	Moderate	—	Hallucinations	(+)
11	Moderate	—	—	(+)
12	Severe	Severe‡	Hallucinations	(+)‡
13	Severe	Severe‡	—	+‡
14	Moderate	Severe	Hallucinations	0
15	Severe	Severe	—	0

*Moderate: ≥ 1.0 to $< 3.0 \times 10^9$ cells/liter; severe: $< 1.0 \times 10^9$ cells/liter.

†0 = no hematuria; (+) = positive BM-test-8 (Boehringer); + = 3–15 rbcs/view field; + + = 16–40 rbcs/view field.

‡Combined with lethal uremia.

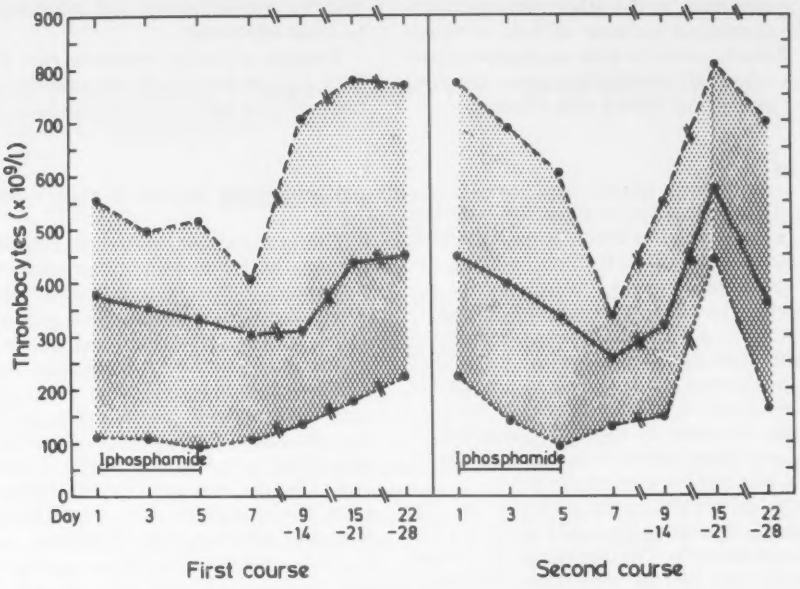

FIGURE 2.—Platelet counts during 2 courses of ifosfamide and mesnum. ———— = mean value, — — — = maximum value, ---- = minimum value.

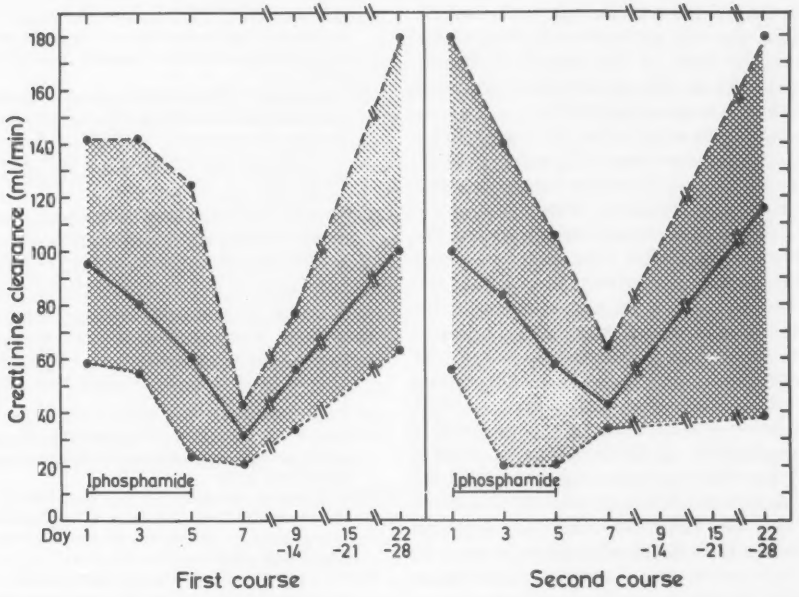

FIGURE 3.—Creatinine clearance during 2 courses of ifosfamide and mesnum. ———— = mean value, — — — = maximum value, —— = minimum value.

pletion of two courses. In the other patients treatment was discontinued because of lack of tumor regression. Even in patients with no change or partial remission after two ifosfamide courses, the drug was stopped because of severe side effects.

DISCUSSION

The present study could not confirm the reported high response rates with ifosfamide in patients with metastatic renal cancer (1,2,4). This may be explained by the differences in the criteria for response, especially in skeletal metastases. Furthermore, in one study (1) irradiation in combination with ifosfamide therapy was given to the metastatic areas. The evaluation of response to ifosfamide should not be based on measurements in such irradiated areas. In order to evaluate the effect of ifosfamide alone, simultaneous irradiation should be given only to some of the metastatic areas.

Using these principles in our patients, we observed one partial remission among 11 patients after two courses of ifosfamide. The partial remission was seen in a patient who had had irradiation. Although the applied radiation dose was rather low, the possibility that it was an important factor in obtaining the partial response cannot be excluded. A nonradiated retroperitoneal tumor in the same patient remained unchanged after treatment with ifosfamide. In addition, the two patients with progressive disease before the start of the second ifosfamide course (early death) should probably be considered as nonresponders (patients 14 and 15).

When starting this study, the urotoxicity-preventing effect of mesnum was still under investigation. It was known, on the other hand, that urotoxicity could, more or less, be avoided by intensive hydration (1). The hydration regimen used and the exact control of the diuretic balance made hospitalization necessary for 11 patients. In spite of the use of mesnum and intensive hydration regimen, two of the 15 patients died from toxic side effects of ifosfamide. In both patients toxic effects on the remaining kidney (uremia) and severe leukopenia were observed before death. In the other patients no severe urotoxicity was observed. Urotoxicity is the main complication of ifosfamide. Our results support the view that mesnum (together with intensive hydration) can effectively prevent it in most patients, as has been shown by other investigators (6–8). Due to the high doses of ifosfamide used in this study, leukopenia was a severe complication resulting in life-threatening infections in eight patients. The cerebral toxic effects (hallucinations) and the severe nausea and vomiting were difficult to treat effectively.

Because of the low response rate and severe toxicity, this study with ifosfamide in renal cancer was discontinued after entering 15 patients.

CONCLUSION

This study shows one partial remission among 15 patients with metastatic renal cancer treated with high doses of ifosfamide with or without irradiation. Whether the partial remission is due to ifosfamide alone or due to the combined effects of ifosfamide and low-dose irradiation remains unclear. Ifosfamide in high doses seems to cause rather high hematologic toxicity. Leukopenia, resulting in life-threatening infections, was the main hematologic side effect in the present study. Urotoxicity can be controlled by mesnum and intensive hydration in most of the patients, but may still represent a severe side effect in some patients.

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Phase I and Preliminary Phase II Observations of High-Dose Intermittent 6-Thioguanine[1,2]

Cary A. Presant,* Alex E. Denes, Leonard Klein, Susan Garrett, and Gerald E. Metter[3]

SUMMARY

6-Thioguanine was administered iv or orally to 66 patients on an intermittent schedule, one dose every 3 weeks. Doses were gradually escalated until moderate toxicity was observed. The dose-limiting toxic effects were myelosuppression and azotemia. The recommended starting doses for phase II or III studies were 700 mg/m² iv and 1400 mg/m² orally. Nephrotoxicity and myelosuppression were reversible in all clearly drug-related instances. Myelosuppression was transient, with nadir blood cell counts observed 10–14 days after drug administration. No cumulative toxicity was observed. Antitumor responses were observed in five of 21 evaluable patients with metastatic colorectal carcinoma including two of four previously untreated patients with that disease. Other than a transient response in a patient with endometrial carcinoma, who received her drug orally, all other responses were observed in patients treated iv with 6-thioguanine. Further phase II trials, particularly in colorectal carcinoma, are recommended.

[Cancer Treat Rep 64:1109–1113, 1980]

6-Thioguanine is an antimetabolite with demonstrated activity in the treatment of acute leukemia. After conversion to 6-thioguanosine monophosphate, tumor cells show decreased synthesis of purine nucleotides and DNA.

6-Thioguanine produces several unusual alterations in cells. When Sarcoma 180 cells are exposed to 6-thioguanine in vitro, they undergo cell surface alterations as a result of inhibition of L-fucose incorporation into membrane glycoproteins (1). This is presumably secondary to decreased GMP formation in the presence of 6-thioguanine. This phenomenon might result in altered tumor growth rates, altered metastatic potential, or altered tissue invasiveness. Furthermore, cultured neuroblastoma cells exposed to 6-thioguanine undergo morphologic differentiation (2), possibly related to decreased cyclic-GMP content.

Although it is an antimetabolite, 6-thioguanine displays unusual kinetics of cytotoxicity in mice bearing transplantable L1210 leukemia (F. Valeriote and T. Vietti, unpublished observations). In contrast to most S-phase-specific agents which produce greater cytotoxicity when given as an infusion, at least as much antileukemic cytotoxicity is produced by 6-thioguanine if given by bolus injection. In addition, bolus injection produces less cytotoxicity against stem cells compared to that produced by infusion of 6-thioguanine. Therefore, bolus injection results in an increased therapeutic index.

It therefore seemed reasonable to explore the clinical use of higher doses of 6-thioguanine given intermittently. Since it had previously been shown (3) that oral 6-thioguanine in small doses had delayed and variable absorption, we anticipated that the iv form would produce biologic effects at lower doses. Compared to equal oral doses, the $C \times t$ product for iv doses was 4.3-fold higher for 6-thioguanine and 1.7-fold higher for 6-thioguanine and its metabolites.

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MATERIALS AND METHODS

Patients were eligible for this phase I and preliminary phase II trial if they had histologically proven malignant disease without bone marrow involvement and not curable by surgery and/or radiation therapy. Furthermore, patients must have become resistant to any effective palliative chemotherapy. Patients were not treated unless they had satisfactorily recovered from prior therapy and had received no chemotherapy for 3 weeks prior to admission to the study. Patients gave voluntary informed consent prior to participation.

Iv 6-thioguanine was supplied by the National Cancer Institute (Bethesda, MD) and was reconstituted in 50 ml of 5% dextrose in water prior to administration. At higher doses, unusual cutaneous reactions were observed, as well as pain along the course of the injection site. Therefore, for higher doses, the 6-thioguanine was diluted to 250-500 ml of 5% dextrose in water and administered over 15–30 minutes. This resulted in improved patient tolerance.

Oral 6-thioguanine was utilized in 40-mg tablets which are commercially available. Ten tablets were ingested every 5 minutes until the total dose had been administered.

6-Thioguanine was administered every 3 weeks. Two or three patients were admitted at any dose level of either iv or oral 6-thioguanine. If no moderate toxicity was observed, patients were entered at the next higher dose level. Once moderate toxicity was observed, three to ten additional patients were treated at that dose level to determine the frequency of toxicity. Patients experiencing no toxic effects at any given dose level could be escalated to the next higher dose level for a subsequent course of therapy.

Some of the patients admitted to the study had measurable tumors. These patients were followed for antitumor response. A partial response was defined as a decrease of at least 50% in the sum of the products of perpendicular diameters of measured tumors, with no tumor increasing in size and no new manifestations of tumor observed. In patients with hepatic metastases, a partial response was defined as a decrease of > 30% in the sum of the distances between the palpable liver edge and the costal margin measured in the midline and both midclavicular lines. Disease progression was defined as a > 25% increase in the sum of the products, or a > 30% increase in liver span. Stable disease was defined as no partial response and no disease progression.

RESULTS

Sixty-six patients were entered in this study. Fifty-nine courses of therapy were administered iv and 35 courses of therapy were administered orally.

The two dose-limiting toxic effects were myelosuppression and renal dysfunction. A summary of the myelosuppression observed is shown in table 1. Although occasional patients had moderate granulocytopenia or thrombocytopenia at doses of < 700 mg/m², these reactions were more frequently observed at iv doses of 700, 800, and 1000 mg/m² of 6-thioguanine. When the characteristics of myelosuppression during the initial course of chemotherapy are separated (table 2), it can be seen that the range of granulocyte and platelet count nadirs fol-

TABLE 1.—Myelosuppression: all courses

Route	Dose (mg/m²)	No. of courses	Median granulocyte count nadir (× 10^3 cells/mm³)	No. of courses < 1500/mm³	Median platelet count nadir (× 10^3 cells/mm³)	No. of courses < 75,000/mm³
Iv	400	12	3.9	1	203	1
	500	7	2.2	1	220	0
	600	8	2.8	0	250	0
	700	15	3.1	2	111	3
	800	14	2.5	2	262	2
	1000	3	2.1	0	365	0
Oral	400	2	3.7	0	168	0
	500	5	4.0	0	175	0
	600	5	5.9	0	290	0
	700	1	9.2	0	315	0
	800	2	6.2	0	301	0
	900	3	5.2	0	184	0
	1000	4	4.2	0	141	0
	1400	8	2.9	1	188	1
	1750	5	2.4	2	263	2
	2000	1	2.6	0	291	0

TABLE 2.—Myelosuppression: initial course

Iv dose (mg/m^2)	No. of courses	Granulocyte count nadir ($\times 10^3$ cells/mm^3)		Platelet count nadir ($\times 10^3$ cells/mm^3)	
		Median	Range	Median	Range
700	11	3.1	0.36–14.0	111	56–389
800	9	1.9	0–5.3	242	16–370

lowing either the 700- or 800-mg/m^2 iv dose was quite broad. The lowest granulocyte count following 700 mg/m^2 was 360/mm^3, and the lowest following 800 mg/m^2 was 0/mm^3. One patient developed septicemia following an iv dose of 800 mg/m^2, but recovered from that complication. The lowest platelet counts were 56,000/mm^3 (after 700 mg/m^2) and 16,000/mm^3 (after 800 mg/m^2). Based upon these data, we would recommend an initial starting dose of 700 mg/m^2 of 6-thioguanine iv with dose escalation permitted to 800 or 1000 mg/m^2 if no toxicity is observed.

Patients with cumulative marrow toxins were able to tolerate a dose of 800 mg/m^2 nearly as well as patients without such prior therapy. The mean granulocyte count nadir in patients with prior nitrosoureas, mitomycin C, or extensive radiotherapy was 2540/mm^3 compared to 3020/mm^3 in other patients.

Higher doses of 6-thioguanine could be administered orally with equivalent toxicity. Frequent myelosuppression was only observed following a dose of 1750 mg/m^2 orally (table 1), and the lowest granulocyte count observed at that dose was 700/mm^3. The lowest platelet count at that dose was 27,000/mm^3. Based upon those data, we would recommend that the initial dose of oral 6-thioguanine be 1400 mg/m^2 with cautious escalation to doses of 1750 and 2000 mg/m^2 if no toxicity is observed.

We were surprised to observe renal toxic effects in many of the patients receiving 6-thioguanine (table 3). Although the mean pretreatment BUN was 15 mg/dl and the mean highest BUN observed during therapy was 31.1 mg/dl, 12 patients had an increase in BUN to > 20 mg/dl. Only four patients had a BUN of > 60 mg/dl at any time during therapy, and all of these patients received iv 6-thioguanine (table 4). The earliest appearance of severe renal toxicity was 48 hours after 6-thioguanine, and the latest appearance was 10 days after drug injection. In three of the four patients, nephrotoxicity was transient with a return to normal renal function within approximately 2 weeks. In one patient, irreversible azotemia was presumably related to hepatorenal syndrome with enlarging liver metastases, but a contribution of 6-thioguanine to the nephrotoxicity could not be excluded. Since none of 11 patients treated initially at an iv dose of 700 mg/m^2 had severe renal toxicity (BUN > 60 mg/dl), while two of nine patients initially treated at an iv dose of 800 mg/m^2 developed that reaction, we feel that the initial dose of iv 6-thioguanine should be 700 mg/m^2. The dose should be escalated only if renal function and myelosuppression permit.

Seven patients receiving iv doses of 6-thioguanine of ≥ 600 mg/m^2 had cutaneous reactions. In one patient, a scratch test with 6-thioguanine was positive. In one patient, the scratch testing was without reaction. In many patients who did not develop cutaneous changes there was severe pain and burning along the course of the injection site. If 6-thioguanine was administered as a more dilute solution, these reactions could be minimized. Premedication with diphenhydramine did not prevent this reaction.

In nine patients, iv or oral dosage was followed by 7–10 days of moderate malaise. In three of these this was described as lightheadedness. One patient had anginal chest pain without electrocardiographic changes and without evidence of infarction. This was not presumed to be related to the 6-thioguanine. One patient had hypotension and diaphoresis

TABLE 3.—Renal toxicity

	Creatinine	BUN
Renal function		
Pretreatment (mg/dl)	0.95	15.1
Highest posttreatment (mg/dl)	1.57	31.1
No. of patients with abnormal test		
BUN > 20 mg/dl or creatinine > 1.5 mg/100 ml	6	12
BUN > 60 mg/dl or creatinine > 3.0 mg/100 ml	3	4
No. of patients with twofold increase	5	8

TABLE 4.—Severe renal toxicity

Dose (mg/m^2)	Route	Pretreatment BUN (mg/dl)	Pretreatment Creatinine (mg/100 ml)	Highest posttreatment BUN (mg/dl)	Highest posttreatment Creatinine (mg/100 ml)	Time to recovery of renal function (days)
600	Iv	14	1.0	74	5.0	12
500	Iv	18	1.5	120	12.5	15
800	Iv	10	1.1	63	2.7	5
800	Iv	15	1.0	132	4.0	*

*Patient had hepatorenal syndrome with progressing hepatic metastases and did not recover.

which lasted 10 minutes and subsided with discontinuation of the drug.

Most patients had mild nausea after iv 6-thioguanine. Fifty percent of patients had some vomiting, although this was of brief duration and easily controlled with antiemetics. Only one patient had severe nausea or vomiting following oral 6-thioguanine. No patient had mucositis, alopecia, or diarrhea.

Forty-four patients were evaluable for antitumor responses following iv 6-thioguanine, and 16 were evaluable following oral 6-thioguanine (table 5). The only response following oral 6-thioguanine was transient; a patient with endometrial carcinoma metastatic to the abdomen and cervical lymph nodes had a partial response lasting only 1 month, with symptomatic improvement during that time.

Following iv 6-thioguanine, the only responses observed were in patients with metastatic colorectal carcinoma. No responses were observed in those with carcinoma of the breast, lung, kidney, pancreas, or uterine cervix, or with malignant melanoma or soft tissue sarcoma. Three of 17 patients resistant to previous 5-FU responded to iv 6-thioguanine. These responses lasted 4, 2, and 2 months. All patients had hepatic metastases. Two of four patients with previously untreated colorectal carcinoma achieved partial remissions each lasting 2 months. Both patients had hepatic metastasis.

One patient previously resistant to 5-FU plus thymidine had hepatic and pelvic soft tissue metastases. Following iv 6-thioguanine, a partial response was rapidly apparent. The patient developed a necrotic abscess in the area of her soft tissue pelvic metastasis and ultimately died of septicemia related to the abscess. At the time of autopsy, no gross residual tumor could be found in the pelvis or in the liver. Microscopic evidence of persistent tumor was, however, apparent.

Another patient had colorectal carcinoma meta-

TABLE 5.—Antitumor responses

Primary tumor type	Iv route: No. of evaluable patients	Iv route: No. of patients with— Partial response	Stable disease	Disease progression	Oral route: No. of evaluable patients	Oral route: No. of patients with— Partial response	Stable disease	Disease progression
Colon, all types (previously untreated)	21 (4)*	5 (2)	7 (1)	9 (1)	4 (2)	—	2 (1)	2 (1)
Breast	4	—	—	4	3	—	—	3
Lung		—						
Small cell	3	—	—	3	1	—	—	1
Other	4	—	1	3	5	—	1	4
Melanoma	2	—	—	2	1	—	—	1
Kidney	1	—	1	—	—	—	—	—
Pancreas	2	—	—	2	—	—	—	—
Sarcoma, soft tissue	4	—	1	3	—	—	—	—
Parotid	2	—	—	2	—	—	—	—
Cervix	1	—	—	1	—	—	—	—
Endometrium	—	—	—	—	1	1	—	—
Hepatoma	—	—	—	—	1	—	—	1

*Nos. in parentheses = No. of previously untreated patients.

static to the liver. The patient had received no prior chemotherapy. After three courses of oral 6-thioguanine, disease progression was apparent. Following iv 6-thioguanine, a partial response was achieved with increased performance status and markedly reduced pain. This response lasted 2 months.

DISCUSSION

Bolus 6-thioguanine repeated every 3 weeks was relatively well-tolerated in the patients studied. Dose-limiting myelosuppression was predictable from prior experiences with oral 6-thioguanine. Although a dose of 700 mg/m^2 is recommended as the initial dose, additional dose escalation may be carried out in many patients if no toxicity is observed. Equitoxic doses were approximately two-fold higher with oral 6-thioguanine, and pharmacologic studies are underway to determine the blood levels obtained with either route of administration. We did not anticipate our observation of mild, reversible renal toxicity following iv 6-thioguanine although in four cases this toxicity appeared to be severe. In three patients there was no evidence of electrolyte or acid-base imbalance, and dialysis was not required. In the other patient, dialysis was not elected due to progression of hepatic metastases. Furthermore, there was no evidence of electrolyte imbalance or disturbance in acid-base regulation.

The cutaneous reactions observed following administration of high doses of iv 6-thioguanine are presumably chemical in nature. Although one patient had a positive scratch test, administration of diphenhydramine did not prevent this reaction in other patients. We presume that the positive scratch test was due to a direct chemical rather than an allergic reaction.

We were surprised to find that iv 6-thioguanine was able to produce a meaningful number of responses in colorectal carcinoma, both in previously treated and previously untreated patients. A prior study of the Eastern Cooperative Oncology Group utilizing a daily oral dose of 1 mg/kg demonstrated partial responses in only one of 17 previously untreated patients and in three of 37 previously treated patients with metastatic colon carcinoma (4).

The encouraging responses observed in colorectal carcinoma warrant further phase II evaluation. A randomized phase II evaluation comparing 5-FU and 6-thioguanine in previously untreated patients with metastatic colorectal carcinoma is currently in progress with the Southeastern Cancer Study Group.

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Phase II Study of Maytansine in Patients With Advanced Lymphomas: An Eastern Cooperative Oncology Group Pilot Study[1,2]

Susan Rosenthal,[3,*] David T. Harris,[4] John Horton,[5] and John H. Glick[6]

SUMMARY

During phase I trials with maytansine some activity against lymphoma and lymphocytic leukemia was noted. Therefore, a phase II trial of maytansine in patients with advanced lymphomas refractory to conventional chemotherapy was begun. There were three partial responders (10%) among 31 patients entered in the trial. Toxicity was acceptable; gastrointestinal and neurologic side effects were the most common. Little myelotoxicity and no hepatotoxicity were observed. We conclude that maytansine has very limited activity in heavily pretreated patients with Hodgkin's disease and non-Hodgkin's lymphomas.

[Cancer Treat Rep 64:1115–1117, 1980]

Maytansine is a naturally occurring ansa macrolide originally isolated from the stem bark of the East African shrub *Maytenus ovatus* (1). Its mechanism of action is similar to that of the vinca alkaloids. Maytansine produces metaphase arrest by interference with mitotic spindle formation caused by inhibition of tubulin polymerization. Unlike vincristine, however, maytansine binds to tubulin irreversibly and produces an irreversible stathmokinetic effect (2,3).

Early clinical trials with maytansine demonstrated acceptable toxic effects including nausea, vomiting, diarrhea, and abnormalities in hepatic function tests (4,5). Little myelosuppression other than sporadic thrombocytopenia, especially in patients with pre-existing liver disease, was encountered (6). Extravasation of the drug caused phlebitis and local necrosis (5). Dose-related neurotoxicity included both central (lethargy, dysphoria, and drop in performance status) and peripheral (paresthesias, jaw pain, muscle weakness, and loss of deep tendon reflexes) effects. Patients with pre-existing vincristine or carcinomatous neuropathies had greater neurologic toxic effects from maytansine (5).

To date, phase II trials of maytansine have shown little activity against breast cancer (7), melanoma (7), lung cancer (8), and colorectal cancer (9). In September 1977, the Eastern Cooperative Oncology Group initiated a phase II trial of maytansine in patients with advanced lymphomas refractory to conventional chemotherapy.

METHODS

All patients had advanced (stage III or IV) histologically documented Hodgkin's disease or non-Hodgkin's lymphoma and were no longer responsive to conventional chemotherapy. Twenty-nine patients had received prior treatment with vinca alkaloids, and one had received VM-26 as well. Many patients had received prior radiation therapy (table 1). Each patient had at least one site of measurable disease. Criteria for ineligibility included chemo-

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TABLE 1.—Pretreatment characteristics of 31 patients with lymphoma treated with maytansine

Characteristic	No. of patients
Histologic type	
Hodgkin's disease	9
Non-Hodgkin's lymphoma	22
Sex	
Males	21
Females	10
Median age in yrs (range): 56 (19–85)	
Performance status	
0–1	11
2–3	17
4	3
Previous treatment	
Chemotherapy only	7
Chemotherapy and radiotherapy	24
Previous exposure to vinca alkaloids	
Vincristine	18
Vinblastine	1
Vincristine and vinblastine	10
Neither	2
Sites of measurable disease	
Lymph nodes	29
Lung	7
Liver	6
Blood and marrow	5
Skin	5
Spleen	1

therapy within 2 weeks of entry in the study and an expected survival of < 2 months. None of the patients was clinically jaundiced, although several had abnormal liver function tests. Pretreatment characteristics of the 31 patients are summarized in table 1.

Maytansine was administered by iv bolus injection over 3–5 minutes at a dose of 0.5 mg/m² daily for 3 consecutive days, with each treatment cycle repeated at 3-week intervals. Patients with pre-existing liver disease and bilirubin or SGOT greater than twice normal received a 50% dose reduction. No dose adjustment was made for low peripheral blood cell counts resulting from prior therapy or disease involvement. Patients continued to receive therapy until disease progression. Those patients who had no change in measurable disease after three cycles of treatment were also considered to have failed maytansine treatment.

Responses were defined by standard Eastern Cooperative Oncology Group criteria. A partial response was defined as a > 50% reduction in the sum of the products of perpendicular diameters of measurable lesions. Disease progression was defined as the unequivocal appearance of any new lesion or an increase of 25% in the product of the perpendicular diameters of any measurable lesion. Responses were recorded only if the observed improvement persisted for > 1 month. Duration of response was measured from the date treatment was started. All patients were considered evaluable for response, even if they received less than one full course of treatment.

RESULTS

Therapeutic Effects

Thirty-one patients were entered in the study. Six patients died within 4 weeks after starting therapy. In each case death was thought to be due to progressive lymphoma and its complications rather than to maytansine treatment. Nineteen patients had progressive disease while receiving maytansine and three had no improvement. Three patients had partial responses; the response rate was 10%. Details about the three responders are shown in table 2.

For each of the responders, treatment was discontinued after three or four cycles even though responses were continuing. In two cases, the patients refused further maytansine because of toxic effects (paresthesias, diarrhea, nausea, and vomiting). In the patient with nodular mixed lymphoma, maytansine was withheld because of thrombocytopenia (30,000–70,000 cells/mm³). Bone marrow involvement was present, however, and it appears likely that the disease, rather than maytansine, was mainly responsible for the thrombocytopenia. No improvement in platelet count was observed after maytansine was discontinued.

The patient with nodular sclerosing Hodgkin's disease responded in his liver and peripheral nodes after one cycle of maytansine. He refused further treatment after the third cycle but remained in good partial remission with return to a normal activity schedule for a total of 13 months without additional therapy. At that point he relapsed in his peripheral nodes and liver and developed ascites.

Toxic Effects

Twenty-nine of the 31 patients were evaluable for toxic effects. The other two patients died within 4 days after starting therapy. Hematologic toxicity was minimal. No patient had a wbc count of < 2500/mm³, and only two patients had wbc counts of < 4000/mm³. No patient had a platelet count of < 30,000/mm³, and only three patients had platelet counts of < 100,000/mm³. No thrombocytopenic hemorrhage or leukopenic infection occurred.

Gastrointestinal side effects were the most common, usually occurring at the end of the treatment

TABLE 2.—Responders to maytansine

Histologic type*	Stage	Previous vincristine/ vinblastine†	Site of response	Duration (mos)
NM	IVB	−/−	Peripheral nodes	4
DPDL	IVA	+/−	Peripheral nodes	4
Hodgkin's disease (NS)	IVA	+/−	Peripheral nodes, liver	13

*NM = nodular mixed lymphoma; DPDL = diffuse poorly differentiated lymphocytic lymphoma; NS = nodular sclerosis.

†No patients with previous VP–16–213 or VM–26 therapy.

cycle or within a few days after treatment. Nine patients had gastrointestinal side effects (nausea, vomiting, and diarrhea), but in only one patient was the toxic effect considered severe. No patient with normal liver function tests at the start of treatment had evidence of hepatotoxicity while receiving maytansine. Eight patients with pre-existing liver function abnormalities had fluctuations in liver function tests while receiving maytansine, but in no patient could deterioration in liver function be clearly ascribed to maytansine treatment. There was no indication of increased neurologic or hematologic toxicity in those patients with pre-existing liver disease who were treated with 50% doses of maytansine.

DISCUSSION

The response rate of the lymphoma patients treated with maytansine in this study was disappointingly low. All of the patients had far-advanced disease and had undergone extensive previous chemotherapy with or without radiation therapy. In most patients maytansine was the third or fourth chemotherapy regimen employed. Most of the patients, however, were in reasonably good condition, and maytansine could be administered in an outpatient setting in almost all instances. Nevertheless, the 10% response rate offers little encouragement that maytansine, at least at this dose and schedule, will be of significant value in the treatment of Hodgkin's disease or non-Hodgkin's lymphoma. Further trials with this agent in patients with lymphoma are not warranted.

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Nerve Conduction Studies in Patients Treated With *cis*-Diamminedichloroplatinum(II): A Preliminary Report[1,2]

Brief Reports and Preliminary Communications

John D. Cowan,* Merrill S. Kies, Joel L. Roth, and Roby P. Joyce[3,4]

SUMMARY

We performed serial histories, physical examinations, and nerve conduction studies on seven cancer patients who were treated with *cis*-diamminedichloroplatinum(II). Total cumulative doses ranged from 200 to 350 mg/m². In nerve conduction studies, three of seven patients showed subclinical, predominantly sensory, changes compatible with a peripheral neuropathy.

[Cancer Treat Rep 64:1119–1122, 1980]

cis-Diamminedichloroplatinum(II) (CDDP) is an anticancer drug introduced by Rosenberg et al (1). It has been found to have clinical utility in the treatment of head and neck, ovarian, bladder, and testicular cancers (2–5). The precise mechanism of action has not been clarified, but it is known to inhibit DNA synthesis by the formation of intra- and interstrand crosslinks in mammalian cell cultures (6), and to be cycle-nonspecific in human cell lines (7).

Unfortunately, the use of CDDP has been complicated by hematologic, audiologic, gastrointestinal, and renal toxicity (8). Since CDDP contains the metal, platinum, which is similar to thallium (9), there is potential for serious nerve toxicity. Furthermore, scattered case reports have appeared in the literature implicating CDDP as a causative agent for peripheral nerve damage in cancer patients exposed to standard doses and schedules (3,10–13). In view of the considerable activity of CDDP and the anticipated development of similar platinum compounds, this study was designed to more precisely evaluate nerve function in patients treated with CDDP by using serial nerve conduction studies as well as the routine history and physical examination.

MATERIALS AND METHODS

From December 1978 to August 1979, ten patients with solid tumors were treated with CDDP as a single agent at the Brooke Army Medical Center, San Antonio, TX. All patients were invited to participate in this study.

Prior to the first CDDP exposure, all patients underwent a neurologic history and physical examination, a battery of laboratory tests including cbc, serum calcium, fasting and 2-hour postprandial blood sugar, VDRL, thyroid function tests, serum folate level, serum B12 level, antinuclear antibodies, rheumatoid factor, urinalysis, and 24-hour urine screen for heavy metals. Nerve conduction studies of the right upper and lower extremities consisting of distal motor latency, distal sensory latency, forearm motor nerve conduction velocity of the ulnar and median nerves, distal motor latency and leg motor nerve conduction velocity of the peroneal nerve, and leg sensory nerve conduction velocity of the sural nerve were performed. Prior to each subsequent dose of CDDP and 2–6 weeks after the last dose of CDDP, repeat history, physical examination, and nerve conduction studies were performed. The nerve conduction studies were considered abnormal if any one of the parameters was greater than two standard deviations from the norm. If a pretreatment parameter was abnormal, the followup measurement was considered changed only if it was unobtainable.

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[2]The opinions or assertions contained within this manuscript are the private views of the authors and are not to be construed as reflecting the views of the Departments of the Army or Defense.

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RESULTS

Seven of ten patients treated with CDDP as a single agent were evaluable. Two patients were excluded because of early death due to their primary tumor and one patient was lost to followup. The evaluable patients consisted of six men and one woman (table 1). The total dose of CDDP ranged from 140 to 305 mg/m². Five of seven patients were treated on Days 1 and 8, every 28 days, with 50 mg/m² of CDDP over 4 hours after hydration; one patient was treated every 21 days with 100 mg/m² over 24 hours after hydration; and one patient was treated every 28 days with 70 mg/m² over 2 hours after hydration and administration of mannitol. The CDDP therapy was discontinued because of disease progression (five patients), renal toxicity (one), and patient request (one).

The pretreatment history and physical examinations showed no neurologic abnormalities in five of seven patients. Patients 5 and 6 had bilateral decreased vibratory sense in the lower extremities. The laboratory screen was negative for all seven patients. Prior to treatment with CDDP, three patients had abnormal nerve conduction findings compatible with peripheral neuropathy. Patient 2 had an unobtainable median and ulnar distal sensory latency. Patient 5 had unobtainable ulnar distal sensory latency, unobtainable sural nerve latency, abnormal median distal sensory latency, abnormal ulnar distal motor latency, abnormal ulnar forearm motor nerve conduction velocity, abnormal peroneal distal motor latency, and abnormal peroneal leg motor nerve conduction velocity. Patient 6 had an unobtainable ulnar distal sensory latency. A summary of the nerve conduction studies is shown in table 2.

The history and physical examination after completion of all CDDP treatment revealed no new clinical peripheral neuropathies. The nerve conduction studies after completion of all CDDP treatment were changed in three patients. Patients 1 and 2 had unobtainable sural nerve latencies. Patient 5 had an unobtainable median sensory latency and a marked prolongation of the peroneal nerve distal motor latency, but normalization of the ulnar distal motor latency.

DISCUSSION

Several reports have associated CDDP usage with peripheral nerve damage. Kedar et al (10) reported a patient who developed a "stocking glove" sensory deficit with nerve conduction study abnormalities after a total dose of 500 mg/m². Three of 100 patients with ovarian cancer treated and reported by Bruckner et al (3,11) developed a peripheral neuropathy after a minimum of 250 mg/m² of CDDP. Von Hoff et al (12) reported three patients with peripheral neuropathy which developed after a dose of 210–510 mg/m². Two of the three patients had nerve conduction studies which disclosed absent sensory nerve action potentials. All three had sural nerve biopsies which demonstrated scattered destruction of the myelin sheaths without axonal disruption. Finally, Reinstein et al (13) recently reported the only case of sequential nerve conduction studies done in a patient treated with CDDP. After treatment with a dose of 700 mg/m² for a malignant fibrous histiocytoma, the patient developed bilateral paresthesias of the hands and feet, with nerve conduction studies revealing a mixed sensory motor peripheral neuropathy of all extremities. No progression in symptoms was reported, but the nerve conduction studies showed further deterioration after CDDP at a total dose of 1206 mg/m².

Although CDDP is implicated as a cause of clinically significant peripheral nerve damage, the case reports are difficult to interpret because: (*a*) pa-

TABLE 1.—Patient profile

Patient No.	Age (yrs)	Tumor type	Previous chemotherapy
1	40	Lung, squamous cell carcinoma	Ifosfamide
2	38	Lung, squamous cell carcinoma	None
3	56	Oropharynx, squamous cell carcinoma	None
4	51	Nasopharynx, squamous cell carcinoma	None
5	53	Nasopharynx, squamous cell carcinoma	Bleomycin, methotrexate, and mitomycin C
6	57	Lung, squamous cell carcinoma	None
7	46	Ovary, adenocarcinoma	None

TABLE 2.—Nerve conduction studies* before and after total dose treatment with CDDP

Patient No.	CDDP Total dose (mg/m²)	CDDP Schedule†	Median nerve DML	Median nerve DSL	Median nerve FMNCV	Ulnar nerve DML	Ulnar nerve DSL	Ulnar nerve FMNCV	Peroneal nerve DML	Peroneal nerve LMNCV	Sural nerve LSNCV
Normal‡	—	—	3.7±0.6	3.2±0.4	57±10	3.2±10	3.2±.50	62±10	< 6	50±12	37±8
1	200	A	3.5/3.3§	2.8/3.0	56/52	2.9/3.0	2.9/3.4	62/63	4.9/4.9	45/42	36/ϕ¶
2	350	A	4.7/4.8	ϕ/ϕ	53/51	2.5/3.6	ϕ/ϕ	57/57	3.8/4.2	48/47	36/ϕ
3	200	A	3.7/3.5	3.2/3.1	54/56	3.5/3.3	3.2/3.2	53/53	4.3/3.2	45/41	41/44
4	300	A	3.5/3.4	3.4/3.5	56/54	3.1/2.8	3.4/3.5	58/58	5.0/4.3	44/43	38/32
5	300	B	5.5/4.7	4.3/ϕ	46/51	4.5/3.5	ϕ/ϕ	47/50	7.9/12.4	30/30	ϕ/ϕ
6	200	A	3.9/3.6	3.6/3.3	51/50	3.4/3.0	ϕ/ϕ	53/51	4.6/4.6	44/43	36/36
7	140	C	3.5/3.5	2.9/3.1	57/54	2.7/3.2	2.9/3.0	57/58	4.9/4.5	43/44	37/41

*DML = distal motor latency (msec); DSL = distal sensory latency (msec); FMNCV = forearm motor nerve conduction velocity m/sec; LMNCV = leg motor nerve conduction velocity (m/sec); LSNCV = leg sensory nerve conduction velocity (m/sec).

†A = 50 mg/m² over 4 hrs after hydration on Days 1 and 8, every 28 days; B = 100 mg/m² over 24 hrs after hydration every 21 days; C = 70 mg/m² over 24 hrs after hydration and mannitol every 28 days.

‡Normal values (mean ± 2 SD) are from the Ohio State University, Department of Physical Medical and Rehabilitation, Columbus.

§Values = pretreatment score/posttreatment score.

¶ ϕ = unobtainable.

tients have been treated with other chemotherapeutic agents known to cause peripheral neuropathy; (*b*) no systematic study has been done to rule out peripheral neuropathy on the basis of nutritional, metabolic, or paraneoplastic phenomena; and (*c*) the peripheral neuropathies have not been consistently assessed by nerve conduction studies.

Motor and sensory nerve conduction and latency studies provide a method to systematically evaluate peripheral nerve damage. They are simple tests, yielding objective and reproducible results in normal subjects (14–16). Nerve conduction and latency studies may demonstrate early sensory nerve damage in cancer patients (17). However, they do not separate the paraneoplastic neuropathic syndrome from the more common noncarcinomatous peripheral neuropathies (18,19).

In our small cohort of patients, nerve conduction studies provided evidence of subclinical peripheral nerve damage after treatment with CDDP to a total cumulative dose of 200–350 mg/m². The changes occurred in one patient with normal pretreatment nerve conduction studies. As noted in the previously reported cases, the damage appeared to be predominately sensory. Our data indicate that CDDP may be a contributing agent to peripheral neuropathies observed in the cancer patient. Patients developing peripheral neuropathies while receiving this drug deserve close monitoring for more serious nerve damage if therapy is to be continued.

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DTIC Therapy in Metastatic Malignant Melanoma: A Simplified Dose Schedule[1]

Brief Reports and Preliminary Communications

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SUMMARY

Ninety-seven patients with metastatic malignant melanoma were treated with DTIC, 850 mg/m² given in single doses at 3–6-week intervals, either alone or in combination with cyclophosphamide (750 mg/m²) and vincristine (2 mg/m²) (DCV). Eighteen patients (19%) had objective disease regression, with a median response duration of 151 days. There was no difference in response rate, response duration, or survival between the group treated with DTIC alone and the group treated with DCV but there was greater gastrointestinal and hematopoietic toxicity in the DCV group. Tumor regression with this single-dose DTIC regimen is similar to that obtained with 5-day courses of DTIC and toxicity is not increased.

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DTIC is still the most effective single agent available for the treatment of metastatic malignant melanoma (1). This agent has traditionally been given in doses of 200–250 mg/m²/day iv over a 5–10-day period and repeated every 3–6 weeks with reported response rates of 17%–22% (2). Because of the relative lack of dose-schedule dependency shown in animal experiments (3), a pilot study using DTIC in intermittent high doses ranging from 650 to 1450 mg/m² given in a single dose iv at 5–6-week intervals was done, resulting in a 20% objective response rate in a group of 20 patients (4). Responses were seen at doses ranging from 750 to 1450 mg/m². In a second group of 20 patients, DTIC (850 mg/m²), cyclophosphamide (750 mg/m²), and vincristine (2.0 mg/m²) (DCV), were all given iv in single doses and repeated at 3–4-week intervals, and a 25% response rate resulted (5). A total of 97 patients with metastatic malignant melanoma have now been treated using DTIC (850 mg/m²) given in single iv doses at 3–6-week intervals, either alone or in combination with cyclophosphamide and vincristine. This report evaluates and compares the results of these two high-dose intermittent DTIC regimens, contrasting them with results obtained using the traditional 5–10-day DTIC schedule.

MATERIALS AND METHODS

Patient Selection

All patients with metastatic melanoma referred to the medical clinic at the Princess Margaret Hospital were considered for study. Any patient considered amenable to surgical cure was referred for surgery rather than being included in this trial. Ninety-seven patients with biopsy-proven malignant melanoma were entered in the study, 57 to the DTIC-alone group and 40 to the DCV group.[4] There were 47 men and 50 women with a median age of 43 years. All 97 patients had metastatic disease which was measurable either directly or radiologically. Of these, 14 had disease limited to skin, lymph nodes, and soft tissue. All other patients had laboratory and/or radiographic evidence of "visceral" involvement (at least one of bone, lung, liver, brain, or gastrointestinal tract). Age, sex, site of primary, and sites of metastases were comparable in the DTIC and DCV treatment groups.

Of these 97 patients, 73 had received some previous treatment in addition to surgery, within 8½ years to 3 weeks of being entered in the study. Fifty-two patients had received local radiotherapy

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[4]Twenty of the DCV-treated patients have been previously reported (ref 5). None of the DTIC-alone group are included in previous publications, except abstracts.

to central nervous system (CNS) metastases, painful bony lesions, or large nodal masses. Three patients had received chemotherapy with agents other than DTIC. Ten patients had received oral bacillus Calmette-Guérin (BCG). One had received both local radiotherapy and chemotherapy with an agent other than DTIC, and seven had received both local radiotherapy and oral BCG. An additional 16 patients received concurrent radiotherapy either to CNS metastases or to painful bone lesions while receiving DTIC or DCV therapy. In all instances where previous or concurrent radiotherapy was given, assessment of DTIC or DCV responses was made on the basis of measurable tumor masses other than those which had been irradiated. Three long-term complete responders to DTIC had oral BCG added to their regimens after 5½, 11, and 15 months in remission with DTIC alone. Any prolongation of remission duration due to BCG would have minimally affected median remission duration in the DTIC group since two of these three patients had already passed the median before BCG was added.

Drug Administration and Dose Schedule

In the DTIC-alone group, DTIC was given every 3–4 weeks as a single iv dose of 850 mg/m². The DCV group received DTIC (850 mg/m²), cyclophosphamide (750 mg/m²), and vincristine (2 mg/m²), all given by single iv dose every 3–4 weeks. Both groups of patients were premedicated with prochlorperazine (Stemetil) or dimenhydrinate (Gravol) and diphenoxylate atropine (Lomotil) and given further doses of these drugs as required over the next 12 hours to control nausea, vomiting, and diarrhea. After the chemotherapeutic agents were given, an iv solution was continued overnight. All patients were hospitalized for 12–24 hours. Repeat courses were given at 3–4-week intervals, unless patients had not recovered from the toxic effects of treatment. Therapy was continued until there was clear evidence of progression of disease. Patients who received even one course were considered evaluable. Of the 97 patients, 33 received one course, 22 received two courses, and 43 received three or more (up to 18) courses. The median number of courses was two for both the DTIC and DCV groups.

Assessment of Response

A partial remission was defined as a > 50% reduction in measurable tumor masses, provided no deterioration of the patient's general condition occurred and no new lesions appeared. A complete remission was the disappearance of all subjective and objective evidence of disease for at least 8 weeks.

RESULTS

Antitumor Effect

The objective response rates with these regimens are summarized in table 1. There was no significant difference in response rate ($P = 0.696$, Fisher's exact test) or median remission duration ($P = 0.087$, median test) between the patients treated with DTIC alone and those treated with DCV. The overall response rate for the two regimens was 19%. Median response duration was 151 days with a range of 81–855 days. The median prolongation of survival among responders (DTIC and DCV combined) was 135 days, which is entirely accounted for by the number of days spent in remission. Survival was significantly improved for responders ($P = 0.037$, Wilcoxon-Gehan test). There was no significant difference in response rate ($P = 0.562$, Fisher's exact test), in remission duration ($P = 0.336$, Wilcoxon-Gehan test), or in overall survival ($P = 0.47$, Wilcoxon-Gehan test) between male and female patients, or among patients with primaries arising in different sites.

Table 2 details the responses of secondaries in each metastatic site, regardless of the response of the disease in the patient as a whole. Metastatic melanoma in bone never responded to therapy, but secondaries in liver and/or lung, as well as in nodes and soft tissue, responded at a rate very comparable to that of the overall disease response rate. Melanoma secondaries in lung showed the best response

TABLE 1.—Antitumor effect of DTIC and DCV therapy

	Response (complete + partial)		Complete response		Remission duration, in days	
Patient group	No.*	%	No.	%	Median	Range
DTIC	10/57	18	4/57	7	181	92–852
DCV	8/40	20	2/40	5	117	83–259
Combined DTIC and DCV	18/97	19	6/97	6	151	83–855

*Values = No. of patients responding/No. in group.

TABLE 2.—Response of metastatic lesions in various sites*

Patient group	Skin ± nodes, ± soft tissue		Lung		Liver		Brain		Bone	
	No.†	%	No.	%	No.	%	No.	%	No.	%
DTIC	7/46	15	5/26	19	5/28	18	0/8	0	0/12	0
DCV	7/36	19	4/11	36	3/20	15	0/6	0	0/7	0
Combined DTIC and DCV	14/82	17	9/37	24	8/48	17	0/14‡	0	0/19‡	0

*Response rate of the secondaries in the listed site, regardless of the response of secondary lesions in other sites.
†Values = No. of patients responding/No. with metastases at the indicated site.
‡3 patients with brain secondaries and 1 with bony lesions had overall responses (all partial) but in none of these 4 patients did the bone or brain metastases per se regress.

TABLE 3.—Toxic effects of intermittent high-dose DTIC therapy (per course)

Patient group (No. of courses)	Nausea		Vomiting		Diarrhea		Neutrophils < 1000		Platelets < 100,000		Flu-like illness		Other (malaise, fever, etc)	
	No.*	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
DTIC (237)	170	72	131	55	48	20	10	4	4	2	4	2	9	4
DCV (112)	95	85	84	75	54	48	32	29	9	8	7	6	8†	7
Combined DTIC and DCV (349)	265	76	215	62	102	30	42	12	13	4	11	3	17	5

*Values = No. of courses with toxic effects.
†Mainly of severe jaw pain which was likely related to vincristine and did not recur when vincristine dosage was reduced to 2 mg/patient rather than 2 mg/m^2.

rate [nine of 37 patients (24.3%)] which was significantly better ($P = 0.03$, Fisher's exact test) than that for bone (none of 19 patients).

Toxicity

Table 3 details the toxic effects seen in this study. The major side effects of both the DTIC and DCV regimens were nausea, vomiting, and diarrhea, which were frequent but mild. Vomiting lasted > 24 hours in only ten of 349 courses of treatment (2.8%), while persistent diarrhea occurred in only one of 349 courses (0.28%). DCV produced vomiting ($P = 0.001$, Fisher's exact test) and diarrhea ($P = 0.0005$, Fisher's exact test) in a higher percentage of patients than did DTIC alone. Severe neutropenia (neutrophils < 1000/mm^3) was uncommon with the DTIC regimen but frequent with DCV ($P = 0.0005$, Fisher's exact test). Significant thrombocytopenia (platelets < 100,000/mm^3) was not common with either regimen, but again was more often seen in the DCV group ($P = 0.009$, Fisher's exact test). No patient in either group had bleeding related to thrombocytopenia, and no serious infections related to neutropenia were seen. There were no treatment-related deaths. The flu-like illness which has been reported with DTIC therapy was infrequent in both groups, but more common in the DCV group ($P = 0.048$, Fisher's exact test). Other side effects such as malaise, fever, and phlebitis occurred infrequently in either group. Severe jaw pain occurred in four of 112 (3.6%) courses of DCV therapy but was always associated with doses of vincristine exceeding 2 mg and disappeared in subsequent courses when the vincristine dosage was reduced to ≤ 2 mg. Severe alopecia occurred in only three patients, all of whom were in the DCV group. It is felt that the alopecia was probably related to cyclophosphamide. All patients were hospitalized overnight for DTIC therapy while this study was underway but patients are now given DTIC according to this schedule as outpatients.

DISCUSSION

Eighteen of 97 patients (19%) had objective disease regression, with a median response duration of 151 days. These results are similar to those obtained with 5-day courses of DTIC (2). The confirmation of this response rate in this larger group of patients supports our earlier suggestion (4) that the lack of dose-schedule dependency reported in animal systems (3) also applies to man. In addition, the

response rate at doses of 850 mg/m² (19%) is similar to that obtained at doses ranging up to 1450 mg/m² (20%), suggesting that the same response rate can be obtained with less toxic doses. The toxic reactions of the group treated with DTIC alone at 850 mg/m² were minimal. When cyclophosphamide and vincristine were added, no gain in response rate or response duration was made, but considerable toxicity was added.

Our work would suggest that DTIC can be given every 3–4 weeks as a single dose in patients with malignant melanoma, with optimal effect and no increase in toxicity. The advantages of this in terms of fewer hospital visits, decreased duration of nausea and vomiting, and better utilization of doctor and patient time are obvious. DTIC is now used in several other malignant diseases (6). Further study of this single-dose form of DTIC therapy could lead to its wider application.

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Failure of Aclacinomycin and 7-Con-*O*-methylnogarol (7-OMEN) to Decrease Cardiac Guanylate Cyclase Activity[1,2,3]

Brief Reports and Preliminary Communications

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The anthracycline antibiotics, doxorubicin (Adriamycin) and daunorubicin induce a variety of cardiotoxic effects, including supraventricular and ventricular arrhythmias, heart block, pericarditis-myocarditis syndrome, and late-onset, dose-related cardiomyopathy which may result in severe, sometimes irreversible, congestive heart failure (1–4). We have recently reported that doxorubicin and daunorubicin selectively decreased the in vitro activity of rat and human cardiac guanylate cyclase (E.C. 4.6.1.2), the enzyme catalyzing the production of guanosine 3′,5′-monophosphate (cyclic GMP) (5). These data suggested that certain aspects of anthracycline antibiotic cardiotoxicity may be related to decreased cardiac guanylate cyclase activity. We therefore thought it important to examine the structurally related analogs aclacinomycin and 7-con-*O*-methylnogarol (7-OMEN), which are thought to have one tenth to one fifteenth the cardiotoxicity of doxorubicin (6,7). The data in the present report show that neither aclacinomycin nor 7-OMEN inhibited cardiac guanylate cyclase in vitro.

METHODS

Aclacinomycin and doxorubicin were dissolved in 25 μl of DMSO and were diluted with water to stock volumes of 10 or 20 mg/ml for use in the experiments; 7-OMEN was dissolved in DMSO alone. The antibiotics were prepared fresh and none altered the pH of the reaction mixture. Controls contained the appropriate amounts of DMSO.

Guanylate cyclase activity was measured as previously described (8). Rat hearts were homogenized in cold 30 mM Tris-HCl, pH 7.6, and spun at 37,000 x *g* in a Sorval refrigerated centrifuge at 4°C for 10 minutes. The supernatant to which aclacinomycin, 7-OMEN, or doxorubicin had been added (immediately prior to incubation) was assayed at 37°C for 10 minutes for guanylate cyclase activity, using a reaction mixture consisting of 20 mM Tris-HCl, pH 7.6; 5 mM $MnCl_2$; 2.67 mM cyclic GMP; a GTP regenerating system (5 mM creatine phosphate, 11.25 units of creatine phosphokinase); 100 μg of bovine serum albumin; 1 mM 3-isobutyl-1-methylxanthine; and 1.2 mM (α-^{32}P)-GTP (International Chemical and Nuclear Corp, Irvine, CA), approximately 5×10^5 cpm. The enzyme preparation (supernatant) contained about 50–200 μg of protein. The reaction was terminated by the addition of 10 μl of 30 mM EDTA, pH 7.6, containing about 30,000 cpm of ^{3}H-cyclic GMP (to estimate recovery in the subsequent steps) and boiling for 3 minutes. After cooling in an ice bath, the (^{32}P)-cyclic GMP formed was isolated by sequential chromatography on Dowex-50-H^+ and alumina using the modification of Krishna and Krishnan (9) and counted in a Packard Tri-Carb liquid scintillation spectrometer. The overall recovery rate of cyclic GMP after the two-stage chromatographic procedure was 90%–95%. Blank (^{32}P)-counting rates averaged 40–50 cpm.

RESULTS

Table 1 shows that doxorubicin (1 mM) decreased rat cardiac guanylate cyclase by approximately 50%–60%. Concentration response curves demonstrate half-maximal inhibition at 0.6–0.8 mM (5). In an attempt to define the structural specificity of the observed effects of doxorubicin on guanylate cyclase activity, we examined the structurally related analogs, aclacinomycin and 7-OMEN, which are thought to have significantly less cardiotoxicity than doxo-

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[3]Doxorubicin, aclacinomycin, and 7-con-*O*-methylnogarol were gifts from Dr. John Douros, National Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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TABLE 1.—Effect of doxorubicin and aclacinomycin on rat cardiac guanylate cyclase activity

Agent	Concentration (mM)	Cyclic GMP (pmols) accumulated/10 mins/mg of protein*
Control	—	110 ± 6
Doxorubicin	1	54 ± 2
Aclacinomycin	0.5	114 ± 3
	1.0	124 ± 5
	2.0	122 ± 3
	4.0	122 ± 6
Nogalamycin	1.0	121 ± 5

*Each value = mean ± SEM of 9 points from 3 hearts except for nogalamycin which = 4 points from 2 hearts.

rubicin and daunorubicin (6,7). Table 1 shows that aclacinomycin at concentrations up to 4 mM did not decrease rat cardiac guanylate cyclase activity. (Concentrations > 4 mM could not be maintained in solution.) 7-OMEN (1 mM) also did not decrease guanylate cyclase activity but again, higher concentrations could not be tested due to insolubility.

DISCUSSION

Our previously reported decrease of rat and human cardiac guanylate cyclase activity in vitro by the anthracycline antibiotics, doxorubicin and daunorubicin (5), appeared to be reasonably specific since guanylate cyclase activity from liver, spleen, kidney, and lung was not decreased by doxorubicin and daunorubicin. Moreover, other antibiotic antitumor agents including streptonigrin, porfiromycin, and mitomycin C did not decrease cardiac guanylate cyclase activity. The present report extends these observations to the potentially important analogs aclacinomycin, which is thought to have one tenth the cardiotoxicity of doxorubicin (6), and 7-OMEN (10), which may have as little as one fifteenth the cardiotoxicity (7). The data clearly demonstrate that aclacinomycin and 7-OMEN did not decrease rat cardiac guanylate cyclase activity in vitro at concentrations at which doxorubicin produced maximal decreases in the activity of this enzyme. These findings appear to further strengthen the possible role of cardiac guanylate cyclase activity in anthracycline antibiotic-induced cardiotoxicity.

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Phase II Evaluation of 4′-(9-Acridinylamino)-methanesulfon-*m*-anisidide (AMSA) in Patients With Advanced Sarcoma[1,2]

Brief Reports and Preliminary Communications

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4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA) is a substituted acridine derivative with activity against a wide range of murine tumors including L1210 leukemia and B16 melanoma (1,2). The mechanism of action of AMSA is felt to be that of the anthracycline antibiotics: intercalation into DNA (3,4). The major toxic effects in phase I studies utilizing a variety of schedules have been hematologic (5–7), with objective responses noted in patients with acute leukemia and carcinoma of the breast (6). In this paper, we report the phase II evaluation of iv AMSA in adult patients with soft tissue or bone sarcomas.

MATERIALS AND METHODS

All patients receiving AMSA had histologically confirmed sarcomas and objectively measurable disease. All 28 patients had received at least one prior polydrug regimen including doxorubicin; in addition, 14 of these patients had received radiation therapy. Tumor histologic types and patient characteristics are shown in table 1.

An adequate trial required at least three doses of AMSA accompanied by dose-limiting blood cell count depression. Therapeutic responses were defined as follows: *partial remission*—a ≥ 50% decrease of all measurable tumors for ≥ 1 month, and *minor response*—an objective tumor decrease (> 25%), but less than that required for a partial remission.

AMSA was administered every 3 weeks by iv injection over 30 minutes. The initial dose was 120 mg/m² and this was escalated to 150 mg/m² if no blood cell count depression occurred. The dose was reduced to 90 mg/m² if the wbc count was 3000–3900/mm³ or if the platelet count was 75,000–99,000/mm³. The drug was withheld for 1 week if the wbc or platelet counts were less than these levels.

RESULTS

Twenty-eight patients were entered in this study. Five patients were judged as having had inadequate

TABLE 1.—Characteristics of 23 evaluable patients

Characteristic	No. of patients
Median age in yrs (range): 46 (18–73)	
Males:females	16:7
Performance status	
50–60	7
70–90	16
Prior therapy	
Chemotherapy only	13
Chemotherapy and radiation therapy	10
Sites of metastases	
Lung	13
Liver	6
Subcutaneous	4
Lymph node	1
Brain	1
Histologic type	
Leiomyosarcoma	6
Synovial cell sarcoma	3
Liposarcoma	3
Hemangiopericytoma	2
Fibrosarcoma	2
Embryonal rhabdomyosarcoma	1
Osteogenic sarcoma	1
Chondrosarcoma	1
Alveolar soft tissue sarcoma	1
Ependymoma	1
Chordoma	1
Mesothelioma	1

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trials. Of these, three patients died of progressive pulmonary disease after receiving one dose of AMSA; two patients refused further therapy.

The toxic effects of AMSA at this dose schedule were mostly hematologic (table 2). Wbc count depression occurred in most patients (nadir range, 900–5400/mm³; median, 2600/mm³). Mild anemia was also noted, especially in patients receiving prolonged therapy (range of hemoglobin decrease, 0.7–5.1 g/100 ml; median, 2.9 g/100 ml). Platelet count depression was infrequent and generally mild (nadir range, 63,000–380,000/mm³; median, 160,000/mm³). Hepatic or renal toxicity was not observed, nor was there any evidence of cardiac toxicity. No patient had mucositis, rash, or alopecia, although mild nausea occasionally occurred.

The 23 adequately treated patients received a median of four doses of AMSA (range, three to 12 doses). One patient with a low-grade fibrosarcoma, a performance status of 80, and previous treatment with only chemotherapy experienced a partial remission consisting of a > 50% decrease in multiple subcutaneous metastases lasting 6 months. No other evidence of antitumor activity was observed.

TABLE 2.—Hematologic toxic effects in 23 patients treated with AMSA

Parameter	No. of patients
Wbc count nadir (cells/mm³)	
≥ 4000	2
3000–3999	7
2000–2999	7
1000–1999	6
≤ 1000	1
Platelet count nadir (cells/mm³)	
≥ 150,000	13
100,000–149,999	7
< 100,000	3
Hemoglobin decrease (g/100 ml)	
0–1.9	5
2.0–2.9	7
3.0–3.9	3
≥ 4.0	8

DISCUSSION

Twenty-three patients with soft tissue or bone sarcoma were adequately treated with AMSA. The drug was well-tolerated, with leukopenia being the most prominent toxic effect. Only one partial response occurred, and this was in a patient with a low-grade fibrosarcoma. Although all patients had received prior chemotherapy, the performance status of the group was generally good and all patients were able to receive AMSA on an outpatient basis. With this number of patients treated, it can be predicted that AMSA has a true response rate of < 20% ($P = 0.05$). It appears that AMSA in this dose schedule has little value as a single agent in previously treated patients with soft tissue sarcomas. No definitive statement can be made concerning the activity of AMSA in the individual histologic types of sarcoma.

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High-Dose Cyclophosphamide Versus Cyclophosphamide, Methotrexate, 5-FU, and Hydroxyurea (CMFH) in the Treatment of Stage III Non-Small Cell Bronchogenic Carcinoma: A Randomized Trial[1]

Brief Reports and Preliminary Communications

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SUMMARY

Fifty-eight patients with non-small cell carcinoma of the lung (33 with epidermoid carcinoma, 22 with adenocarcinoma, and three with large cell anaplastic carcinoma) were treated with high-dose cyclophosphamide (CTX) or, alternatively, with CTX, methotrexate, 5-FU, and hydroxyurea (CMFH) in a controlled study. Two partial remissions were achieved (one in each regimen [3%]). Seventeen of 29 patients treated with CTX and 14 of 29 patients treated with CMFH showed no change (differences were not statistically significant). Toxicity was moderate in both regimens. Median survival was 24 weeks for patients treated with CTX and 26 weeks for patients treated with CMFH (differences were not significant). The results show that the therapeutic activity of CMFH is not higher than that of CTX alone.

[Cancer Treat Rep 64:1131–1134, 1980]

Survival of patients with advanced lung cancer treated with single-agent chemotherapy is around 4–5 months. Combination chemotherapy seems to be more effective in the treatment of oat cell carcinoma (1). The use of COMB (cyclophosphamide [CTX], vincristine, methyl-CCNU, and bleomycin) aroused particular interest. Livingston et al (2) reported that COMB-treated epidermoid carcinoma showed an objective remission rate of 33%. These results were not confirmed by Bodey et al (3) who showed that the activity of COMB was not more effective than that of high doses of CTX alone.

Consequently, after showing in a previous study (4) that CMFH (CTX, methotrexate, 5-FU, and hydroxyurea) was well-tolerated by patients with advanced carcinoma of the lung, we carried out a prospective randomized study comparing CMFH with CTX alone at high doses, as previously done for COMB by Bodey et al (*see* above).

MATERIALS AND METHODS

Patients with a microscopically confirmed diagnosis of stage III M_0–M_1 (5) non-oat cell bronchogenic carcinoma that was either untreatable by radiotherapy or recurred after it were included in the trial if they had progressive disease and their prognosis for survival was > 2 months. Patients who were previously treated with chemotherapy or had received other antitumor treatment < 1 month before were not included. Other eligibility criteria included: good bone marrow, liver and renal functions, performance status (PS) (6) > 40%, age < 75 years, and absence of chronic non-neoplastic diseases. Patients were randomly assigned to the two regimens according to the following stratification criteria: histologic type (7), PS (level A: 40%–60%, level B: 70%–100%), extent of disease (8), and sex. The regimens were: CTX at a dose of 1000 mg/m² iv every 21 days versus CMFH, ie, CTX at a dose of 10 mg/kg iv and 5-FU at a dose of 15 mg/kg iv on Day 1, methotrexate at a dose of 0.05 mg/kg sc (divided in three daily doses), and hydroxyurea at a dose of 20 mg/kg orally from Day 4 to Day 7. The course was repeated starting from Day 10 and was continued as long as it was tolerated (wbc count < 4000/mm³, platelet count < 100,000/mm³, or other severe side effects).

After two courses of both regimens (in any case within the 3rd month from the beginning of treat-

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TABLE 1.—Clinical characteristics

	CTX group	CMFH group
Total evaluable patients	29	29
Males	24 (83%)	27 (93%)
Females	5 (17%)	2 (7%)
Median age in yrs (range)	57 (46–75)	61 (48–72)
No. of patients with PS—		
40%–60%	18	16
70%–100%	11	13
Median PS (range)	60% (40%–90%)	60% (40%–80%)
Median No. of therapy courses (range)	3 (2–10)	2 (1–5)
Median length of treatment in days (range)	60 (30–500+)	75 (30–360+)
No. of patients with—		
Microscopic diagnosis		
Histologic	16 (55%)	16 (55%)
Cytologic	10 (34%)	9 (31%)
Both	3 (10%)	4 (14%)
Epidermoid carcinoma	18 (62%)	15 (52%)
Adenocarcinoma	10 (34%)	12 (41%)
Anaplastic large cell carcinoma	1 (3%)	2 (7%)
Extent of disease		
Stage III M_0	13 (45%)	12 (41%)
Stage III M_1	16 (55%)	17 (59%)
Limited	16 (55%)	15 (52%)
Extensive	13 (45%)	14 (48%)

ment) clinical evaluation was made. The evaluation criteria were the following: *complete remission (CR)*—the disappearance of all measurable lesions; *partial remission (PR)*—a > 50% reduction of all measurable lesions or > 75% reduction of all evaluable lesions (atelectasis or pleural effusion); *no change (NC)*—no change in measurable lesions (or reduction between 25% and 50%) or in evaluable lesions for at least 1 month; and *disease progression*—an increase in lesion size of > 25% or the appearance of new lesions. Patients with CR, PR, or NC were considered to be responders; patients with disease progression were considered to be nonresponders. The chi-square test was the statistical method used to compare the groups. Survival was calculated by the actuarial method.

RESULTS

The characteristics of the objectively evaluable patients are shown in table 1. In table 2 the overall response rate shows that no statistical difference exists between the two regimens.

Considering objective response (PR plus NC) according to histologic type, epidermoid carcinoma was more responsive to CTX (13 of 18 patients, 72%) than to CMFH (eight of 15 patients, 53%), while adenocarcinoma was more responsive to CMFH (seven of 12 patients, 58%) than to CTX (four of tèn patients, 40%); no significant difference was noted.

Because of the small number of patients with large cell carcinoma, a comparison between data relating to these patients was not feasible. Considering the objective remission according to PS (PS of 40%–60%—CTX, 50% and CMFH, 56%; PS of 70%–100%—CTX, 82% and CMFH, 46%) and the extent of disease (limited: CTX, 75% and CMFH, 53%; extensive: CTX, 46% and CMFH, 50%), no significant differences could be noted. There was no statistically significant difference in the survival of patients treated with CTX (median, 24 weeks; range, 6–65+ weeks) compared to that of those treated with CMFH (median, 26 weeks; range, 9–100+ weeks). With both regimens, responders lived longer than nonresponders (fig 1) (CTX = $P < 0.05$ at 24 weeks; CMFH = $P < 0.05$ at 16 and 24 weeks and $P < 0.01$ at 20 weeks). Patients with epidermoid carcinoma had a longer median survival when treated with CMFH than when treated with CTX (32 versus 25 weeks, $P < 0.05$). Patients with extensive epidermoid carcinoma appeared to have a longer median survival with CMFH than with CTX (35 versus 22 weeks, respectively), and patients with limited epidermoid carcinoma had a median survival of 26 weeks with both regimens.

TABLE 2.—Overall objective response rate

Response	No. of patients	
	CTX group (29 patients)	CMFH group (29 patients)
CR	0	0
PR	1 (3%)	1 (3%)
NC	17 (59%)	14 (48%)*
PR + NC	18 (62%)	15 (52%)*
Disease progression	11 (38%)	14 (48%)
Median length of PR + NC in wks (range)		
CTX group: 15 (9–52)		
CMFH group: 20 (4–60)		

*No significant difference.

Considering the median overall survival according to PS (PS of 40%–60%—CTX, 22 weeks and CMFH, 26 weeks; PS of 70%–100%—CTX, 30 weeks and CMFH, 26 weeks) and the extent of disease (limited: CTX, 22 weeks and CMFH, 26 weeks; extensive: CTX, 22 weeks and CMFH, 24 weeks), no statistically significant difference existed between the two regimens.

Leukopenia, nausea, vomiting, and anorexia were the main side effects. Treatment was discontinued because of leukopenia in ten of 29 patients (34%) receiving CTX and in 21 of 29 patients (72%) receiving CMFH ($P < 0.01$). Such a difference was predictable, however, since CMFH was to be continued until the appearance of leukopenia or other side effects.

CONCLUSIONS

Only our patients with epidermoid carcinoma are suitable for comparison with similar treatment groups reported in the literature. Though remission rates were similar, we obtained a higher median survival compared with the previously mentioned

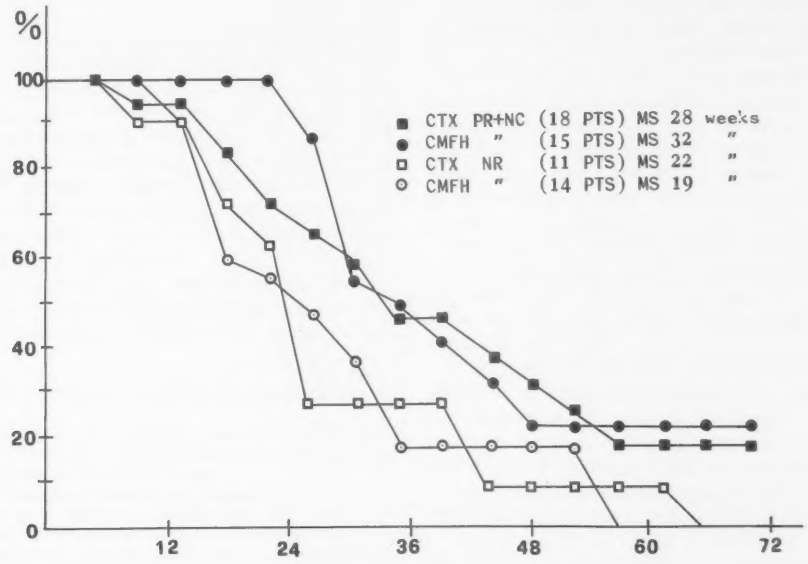

FIGURE 1.—Survival related to objective response. MS = median survival; NR = nonresponders.

patients of Bodey et al (3) (CTX, 14.4 weeks and COMB, 10.9 weeks). Other investigators reported mean survival times similar to those reported by us: Bitran et al (9) with CAMP (CTX, doxorubicin, methotrexate, and procarbazine), 28 weeks and Chahinian et al (10) with MACC (methotrexate, doxorubicin, CTX, and CCNU), 30 weeks. The percentage of remissions obtained by us with CTX alone is similar to that obtained by Bodey et al and does not differ from either that obtained with our CMFH regimen or that obtained by Bodey's COMB regimen.

The results indicate that the therapeutic activity of CMFH is not higher than that of CTX. Only survival data relating to epidermoid carcinoma show a significant improvement with CMFH. However, the small number of our patients suggests further studies. Considering that CMFH toxicity is acceptable, we conclude that its use is justifiable only in patients who do not respond to other well-known regimens.

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VP-16-213 in Combination With Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone in the Treatment of Non-Hodgkin's Lymphomas

Brief Reports and Preliminary Communications

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SUMMARY

VP-16-213 has been used in 5-day courses together with cyclophosphamide, doxorubicin (Adriamycin), vincristine, and prednisone for treatment of 74 consecutive patients with non-Hodgkin's lymphomas. Ninety-one percent of patients with favorable histology and 69% with unfavorable histology achieved complete or partial remissions (overall response rate of 76%). The frequency of complete remission was disappointingly low (22%), possibly due to reduction of doses of the other myelosuppressive agents in the present combination. Further trials with VP-16-213 in other combination chemotherapy are required.

[Cancer Treat Rep 64:1135–1137, 1980]

Since the early 1970s, the management of patients with advanced non-Hodgkin's lymphoma (NHL) has been based on combination chemotherapy utilizing cyclophosphamide, vincristine, and prednisone (COP). These regimens, regardless of dose and schedule, have produced an overall response rate (complete or partial remissions) of 60%–90% (1–3). Despite this high initial response rate, most of the patients eventually relapse and there is a strong need for improved regimens.

The most important new drug in the chemotherapy for NHL is doxorubicin (Adriamycin). Used as a single agent, it has produced a 40% response rate (4); used in combination with the COP regimen (CHOP), it has produced a 50%–70% complete remission frequency (5).

Another new drug is the epipodophyllotoxin derivative VP-16-213; in several studies it has demonstrated significant activity in patients with NHL, with an overall response rate of approximately 25% in previously treated patients (6,7). The place of VP-16-213 in the combination chemotherapy for NHL has not yet been established.

The present study was designed to test the activity and patient tolerance of a new combination consisting of COP with the addition of doxorubicin and VP-16-213.

MATERIALS AND METHODS

The study was carried out as a collaborative, nonrandomized, phase II study among three Cancer and Leukemia Group B member institutions. Consecutive patients with a histologically verified diagnosis of NHL subclassified according to the Rappaport classification (8) were entered in the study. The study was primarily for previously treated patients, but since protocols of higher priority were closed to patient entry, untreated patients were also entered in the latter part of the study.

Staging procedures included lymphography and percutaneous liver and bone marrow biopsies. Most patients had stage III–IV disease (Ann Arbor classification [9]); four patients with stage IIB disease were also included.

The treatment schedule consisted of the iv administration of cyclophosphamide (300 mg/m^2), doxorubicin (30 mg/m^2), and vincristine (1.4 mg/m^2; maximum, 2 mg) on Day 1 and the oral administration of VP-16-213 (40 mg/m^2/day) and prednisone (40 mg/m^2/day; in some cases, 100 mg/m^2/day) on Days 1–5. The treatment cycle was repeated every 3 weeks with adjustments in dose according to the toxicity. In the absence of toxicity a 30% increase in the dose of cyclophosphamide, doxorubicin, and VP-16-213

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was prescribed. A minimum of three treatment cycles were required unless the patient had rapidly progressing disease. Patients achieving complete remission (CR) or partial remission (PR) usually received a total of 12 cycles.

CR was defined as the complete disappearance of all measurable parameters of disease; PR was defined as a > 50% reduction in tumor size.

RESULTS

Eighty-eight patients were entered in the study, but in 14 the treatment effect could not be evaluated because of other concurrent therapy (ten patients), protocol violation (two), lack of measurable tumor (one), or early death (one). The results of treatment of 74 evaluable patients are shown in table 1. Sixteen patients (22%) achieved a clinical CR, 40 patients (54%) achieved a clinical PR. The response frequency (CR plus PR) was higher in the group with favorable histology (well-differentiated lymphocytic lymphomas, nodular poorly differentiated lymphocytic lymphomas, and nodular mixed lymphomas) than among patients with unfavorable histology (histiocytic lymphomas, diffuse poorly differentiated lymphocytic lymphomas, diffuse mixed lymphomas, and diffuse undifferentiated lymphomas). In the latter group 27 patients had diffuse histiocytic lymphomas and six of the 27 achieved a CR.

Previously untreated patients did better than previously treated patients. The range of response frequency (CR plus PR) varied from 100% among untreated patients with favorable histology to 62% in previously treated patients with unfavorable histology. This difference was also reflected in the duration of response and in survival. However, it is noteworthy that 11 of 18 patients who previously had received doxorubicin or cyclophosphamide in various combinations responded to the present combination.

Three of the four patients with stage IIB disease belonged to the group of untreated patients with unfavorable histology. All three patients achieved a CR and two of them are still in CR at 1½ years.

Doses were escalated in ten patients. The response frequency was the same as that found in patients with no dose escalation.

The various forms and grades of toxicity are shown in tables 2 and 3. Sixteen patients developed hematologic toxic effects (grade 3 or 4): nine of the 16 had received other antineoplastic treatment prior to the present study, 13 developed the severe toxicity following standard doses of the regimen, and only three had received increased doses.

Among 12 patients with massive infections, three died with drug-induced granulocytopenia. Six of the 12 patients (one of the three who died) had received no prior therapy.

DISCUSSION

In our series of patients with NHL the addition of VP-16-213 to the CHOP regimen with concurrent reduction of the doses of the other myelosuppressive agents led to a good overall response, considering the fact that many of the patients previously had received combination chemotherapy and radiotherapy. However, the percentage of patients achieving CR was not satisfactory, especially in the group receiving no prior treatment. In comparison with the reported results of the CHOP regimen (5),

TABLE 1.—Results of treatment with VP-16-213 in combination with cyclophosphamide, doxorubicin, vincristine, and prednisone in 74 patients with NHL

Patient group*	No. of patients	No. of patients achieving—				% CR + PR	Median duration of CR (days)
		CR	PR	NC†	PD†		
Untreated							
Favorable histology	14	4	10	0	0	100	360+
Unfavorable histology	30	9	13	4	4	73	290+
Total	44	13	23	4	4	82	
Previously treated							
Favorable histology	9	2	5	1	1	78	123, 291+
Unfavorable histology	21	1	12	4	4	62	480+
Total	30	3	17	5	5	67	

**Favorable histology*: well-differentiated lymphocytic lymphomas (4 patients), nodular poorly differentiated lymphocytic lymphomas (15), and nodular mixed lymphomas (4). *Unfavorable histology*: histiocytic lymphomas (28 patients), diffuse poorly differentiated lymphocytic lymphomas (7), diffuse mixed lymphomas (5), and diffuse undifferentiated lymphomas (11).

†NC = no change; PD = progressive disease.

TABLE 2.—Definition of toxicity

Grade	Granulocytes/μl	Platelets/μl	Gastrointestinal effects	Neurologic effects
0	≥3500	>200,000	None	None
1	>2000–3500	>100,000–200,000	Mild (eg, nausea, dyspepsia)	Paresthesia and/or absent deep tendon reflex
2	>1000–2000	>50,000–100,000	Moderate (eg, vomiting)	Mild muscular hypofunction (eg, slight weakness in legs)
3	≥500–1000	≥25,000–50,000	Pronounced (eg, hemorrhage)	Clear muscular hypofunction (eg, drops things, needs cane)
4	<500	<25,000	Life-threatening (eg, massive hemorrhage, ileus)	Severe paresis (eg, cannot walk unassisted)

TABLE 3.—Toxic effects of treatment with VP–16–213 in combination with cyclophosphamide, doxorubicin, vincristine, and prednisone

	No. of patients with toxicity grade*—				
Type of toxic effects	0	1	2	3	4
Hematologic	10	27	21	11	5
Gastrointestinal	44	13	5	1	2
Neurologic	33	20	18	1	0

**See* table 2.

the present regimen therefore did not seem to be of significant therapeutic benefit.

The explanation for the low frequency of CR is only speculative. It may be due to the lowering of the doses of doxorubicin and cyclophosphamide caused by the addition of the new myelosuppressive agent VP-16-213. In fact, combined myelosuppressive effect was moderate to severe in half the patients, but in the other half only mild or no hematologic toxic effects were elicited; in retrospect, the doses probably could have been increased in this latter group. Another possibility is some type of antagonism between VP-16-213 and one or more of the other drugs. Indeed, preliminary results from one trial suggest such an antagonism between VP-16-213 and cyclophosphamide (10).

Further investigations with VP-16-213 in other combinations are required before a final statement of the value of this drug in combination chemotherapy for NHL can be made.

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Mitomycin C and Bleomycin in the Treatment of Far-Advanced Cervical Cancer: A Southwest Oncology Group Pilot Study[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Under the auspices of the Southwest Oncology Group, Wayne State University tested a schedule using bleomycin and mitomycin C that was previously reported to give a response rate of 88%. In our patients the same regimen produced similar toxic effects but a far different response rate of 15.8% (one complete remission and two partial remissions). The only discernible difference in the two patient groups was the amount of radiation given prior to chemotherapy.

[Cancer Treat Rep 64:1139–1140, 1980]

Initial therapy for metastatic squamous cell carcinoma of the uterine cervix remains experimental. Recently, research interest has centered on bleomycin and mitomycin C for treatment of this disease (1–4). Baker et al (5,6) reported a 39% response rate using mitomycin C, vincristine, and bleomycin. That study also suggested that the schedule of bleomycin directly influenced toxicity and response. Miyamoto et al (7,8) recently described a series of 15 patients showing a response rate of 88% using mitomycin C (10-mg bolus) on Day 8 and low-dose bleomycin (5 units/day) as a 7-day continuous infusion in disseminated cervical cancer. The cycle was repeated on alternate weeks for a total of six courses; maintenance therapy was also described. We designed a Southwest Oncology Group pilot study to determine if Miyamoto's results could be duplicated.

MATERIALS AND METHODS

Patients entered in this study had microscopically proven, incurable, local recurrence or distant metastatic squamous cell cancer of the uterine cervix. Only those with measurable disease were eligible for study. Patients were required to have a life expectancy of at least 6 weeks and no active site of infection. Pretreatment evaluation included physical examination, laboratory evidence of adequate renal function (BUN $\leq$ 20 mg/100 ml, creatinine $\leq$ 1.5 mg/100 ml, creatinine clearance $\geq$ 70 ml/min), wbc count $\geq$ 4000/mm^3, and platelet count $\geq$ 100,000/mm^3. All patients were required to have pulmonary function testing prior to therapy, and diffusion studies were to be within 70% of predicted normal. Patients previously treated with either mitomycin C or bleomycin were ineligible for study. Standard definitions of complete and partial response were used.

Nineteen eligible patients were entered in the study. Patients who completed two courses of bleomycin and mitomycin C were considered evaluable. Seventeen of the 19 patients had had no prior chemotherapy; 18 of 19 patients had had prior pelvic radiation therapy.

RESULTS

All 19 patients who were entered in the study were evaluable for response and toxicity. Three of 19 patients (15.8%) responded to this regimen with a 95% confidence interval and a true response rate

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of 3.38%–39.58%. Two patients (10.6%) achieved partial responses lasting 10 and 25+ weeks, respectively, and one (5.3%) achieved a complete response lasting 22 weeks. Of the partial responders, one patient had measurable pulmonary metastases and the other had bladder metastases recognized by cystoscopy. The patient who completely responded had pulmonary lesions and had not received prior radiation therapy.

Toxicity

Twelve of 19 patients received between four and six of the total of six planned courses. Median nadir wbc count fell from 6100/mm³ for those patients who received two courses to 3500/mm³ for the three patients who received the full six courses. Median nadir platelet count fell from 330,000/mm³ to 52,000/mm³ in those receiving two and six courses, respectively. One patient, with initially normal pulmonary function, showed a clear-cut decrease in ventilatory function and diffusion capacity. Eight patients had either mild or moderate pulmonary dysfunction at the start of therapy; none of this group became measurably worse while in the study. Clinical toxic effects included mucositis (seven patients [two severe]; 36.8%), nausea and vomiting (five; 26.3%), alopecia (three; 15.8%), fever and chills (two; 10.5%), weakness and tightness of skin (two), and diarrhea, anorexia, and dyspnea (one each; 5.3%).

DISCUSSION

The relatively mild toxicity resulting from the use of bleomycin infusion and mitomycin C in advanced uterine cervical cancer, as reported by Miyamoto et al (7,8), has been confirmed by this study. We cannot, however, confirm their reported response rate. The only discernible difference noted between the two populations studied was the dose of prior radiation therapy administered to each group: Miyamoto's patients were uniformly given 3000 R to the pelvis prior to chemotherapy while our patients generally had received > 5500 R. We have no reason to suspect that this would lower our response rate. We also note that while eight of our patients had signal lesions that were difficult to measure (three by ultrasound, two by rectal examination, one para-aortic node, one bony lesion, and one lymphangiogram), 11 of 19 patients had easily measurable and reproducible lesions in the lung (4), reproducible pelvic mass (3), vaginal wall (2), or superficial lymph nodes (2). The median Karnofsky performance status of patients in our study was 75%, suggesting that the clinical status of the vast majority of our patients permitted a satisfactory phase II trial.

Miyamoto reported that all responses started within the first two courses of therapy. In our study, no patient received less than two courses and those responding in our study did so within two courses. The median number of courses was 4.0 in the 16 who failed.

Miyamoto's work reporting a response rate between 88% and 95% for the combination of mitomycin C and bleomycin could not be duplicated in our patients. Unfortunately, for us, the schedule proved to be cumbersome, with protracted and repeated hospital stays, and failed to achieve the results of larger clinical studies (5,6) utilizing a less demanding schedule.

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Phase II Trial of Vindesine in Advanced Head and Neck Cancer[1,2]

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SUMMARY

Twenty-one patients with advanced epidermoid carcinoma of the head and neck region were treated with vindesine. Therapy was started at a dose of 3 mg/m². Dose escalation by 0.5 mg/m² weekly to a maximun of 4.0 mg/m² was permitted when no toxicity was seen. Major dose-limiting toxic effects were neutropenia and peripheral neuropathy. Objective responses were seen in five patients: three partial responses lasting 2, 2, and 3 months, and two minor responses lasting 2 and 5 months.

[Cancer Treat Rep 64:1141–1142, 1980]

Vindesine is a semisynthetic vinca alkaloid derived from vinblastine sulfate. Phase I evaluation demonstrated that its dose-limiting side effects included myelosuppression and neurotoxicity (1). Other reactions such as myalgias, alopecia, and phlebitis have been reported (2). Rapid iv injection of 3–4 mg/m² every 1–2 weeks was found to produce manageable toxicity. For the past 3 years, phase II trials of this agent have been conducted in a variety of neoplasms at Memorial Hospital. Objective responses have been observed in patients with lung cancer, breast carcinoma, melanoma, esophageal carcinoma, renal cell carcinoma, testicular carcinoma, soft tissue sarcoma, mesothelioma, Hodgkin's disease, non-Hodgkin's lymphoma, acute lymphoblastic leukemia, and acute myelocytic leukemia (3). The present study was designed to evaluate the usefulness of this agent in advanced head and neck cancer.

METHODS AND MATERIALS

Between October 1977 and May 1979, 22 patients with histologically proven epidermoid head and neck cancer were entered in the trial. All had complete physical examination, 12-channel screening profile, cbc including platelet counts, chest roentgenograms, and scintiscans when indicated.

Therapy was started at a dose of 3 mg/m² of vindesine (supplied by Eli Lilly and Co, Indianapolis, IN) given by rapid injection through an established free-flowing iv line. Drug doses were repeated at weekly intervals until either dose-limiting toxicity or response was achieved. When such toxicity was attained, appropriate reduction in dose was employed in subsequent treatments which were continued until progression of disease. When no toxicity was achieved, doses were escalated in most cases by 0.5 mg/m² at weekly intervals to a maximum of 4.0 mg/m²/week. Of the 22 patients who started to receive treatment, all were evaluable except one who was lost to followup after one dose.

Partial response was defined as a decrease of at least 50% of the sum of the two greatest perpendicular diameters of all measurable lesions for at least 1 month. A minor response represented objective tumor decrease less than that required for partial remission.

As shown in table 1, the median initial performance status was 80%. Over 80% of the patients had had prior radiation therapy, 50% had had prior surgery, and about one third had had prior chemotherapy. Most patients had disease recurrence at the primary site and/or in the neck at the time of treatment; 50% of the patients had disease recurrence in the lung.

RESULTS

As shown in table 2, most of the patients had three or more doses of vindesine. The major dose-limiting toxic effects were neutropenia and peripheral neuropathy. A decrease of platelet count was seen in three patients, but in all cases the absolute platelet counts were > 150,000/mm³ and, therefore, of no clinical consequence. Three patients experi-

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Median age in yrs (range): 61 (32–73)	
Sex	
Men	14
Women	7
Median Karnofsky performance status (range): 80 (50–100)	
Prior therapy	
Total	21
Surgery only	1
Radiation therapy only	4
Surgery + radiation therapy	9
Surgery + chemotherapy	1
Radiation therapy + chemotherapy	3
Surgery + radiation therapy + chemotherapy	3
No prior chemotherapy	14
Primary sites	
Oropharynx	6
Oral cavity	5
Larynx	4
Pyriform sinus	2
Nasopharynx	4
Site of measurable disease	
Primary	5
Neck	7
Skin	1
Bone	3
Lung	11

enced a partial response lasting 2, 2, and 3 months, respectively. Responding sites were in neck nodes, lungs, and subcutaneous tissues. Two other patients experienced minor responses lasting 2 and 5 months. All responders had a performance status of > 60%. Although no relapses occurred after dose reductions, one relapse occurred in a partial responder

TABLE 2.—Response to therapy and toxic effects

	No. of patients
No. of vindesine courses	
2	3
3	3
≥ 4	15
Toxic effect from treatment	
Wbc count nadir (cells/mm^3)	
3000–3900	3
2000–2900	9
< 2000	3
Platelet count nadir (cells/mm^3)	
< 150,000	0
Paresthesia	12
Constipation	9
Response to therapy	
Partial	3
Minor	2
Progression	16

TABLE 3.—Characteristics of responders

Characteristic	No. of responders	
	Partial	Minor
Primary sites		
Oral cavity	3	1
Oropharynx	0	1
Prior radiation therapy	2	1
Prior chemotherapy	1	0

when the treatment had to be discontinued after 2 months of therapy because of severe neuropathy. The characteristics of responders are shown in table 3.

DISCUSSION

The single-agent data on the activity of the vinca alkaloids in head and neck cancer are very limited; one disease-oriented phase II trial of vinblastine demonstrated that the drug had significant activity (objective responses in 11 of 23 evaluable patients) but also that the median duration of responses was only about 2 months (4). A trial of vindesine was, therefore, of great interest to us. The patients in the present study had a high median performance status, and 14 of the 21 patients had had no chemotherapy prior to vindesine. Nevertheless, we observed only three very brief partial responses and two minor responses. Thus, the activity of this agent appears disappointing, especially when compared with the two agents with generally recognized activity in head and neck cancer (5,6). We conclude that vindesine used singly has no important therapeutic role in this dose and schedule in patients with epidermoid carcinoma of the head and neck.

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Combination Chemotherapy for Advanced Colorectal Cancer With Triazinate, ICRF-159, 5-FU, and Methyl-CCNU[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Twenty-four patients with advanced metastatic colorectal cancer with measurable sites of disease were treated with a combination of 5-FU, methyl-CCNU, ICRF-159, and triazinate. Only two had objective regression of disease. Two patients died of complications from drug-induced myelosuppression. Toxic effects were moderate in the remaining patients. Attempts to escalate doses were limited by myelosuppression. The regimen used has no advantage over 5-FU alone. It is unlikely that triazinate and ICRF-159 will add substantially to the efficacy of chemotherapy for colorectal cancer.

[Cancer Treat Rep 64:1143–1145, 1980]

Although three studies in advanced colorectal cancer have demonstrated the addition of methyl-CCNU to improve the response rate over that obtained with 5-FU alone, the extent of this improvement is minor, and impact on survival is not apparent (1). Two new agents, triazinate (TZT, Baker's antifol) and ICRF-159 (an analog of EDTA, Rasoxane), have response rates between 14% and 20% in patients who have failed prior chemotherapy with 5-FU (2,3). The present study was an attempt to develop a tolerable combination of 5-FU, methyl-CCNU, and the two newer agents.

MATERIALS AND METHODS

Eligibility was restricted to patients with metastatic or locally recurrent carcinoma of the colon or rectum beyond hope of surgical eradication. A measurable area of known malignant disease was required in order to adequately assess response. Patients were excluded if they had undergone any prior chemotherapy or whole-abdominal radiation therapy; were severely malnourished; were fully bedridden; or had a serum creatinine of > 1.5 mg/dl), a wbc count of $< 4000/\mu l$, or a platelet count of $< 100{,}000/\mu l$. Prior surgical procedures had to have been done > 3 weeks before the study, and patients had to be free of active infection.

Treatment was administered on a 28-day cycle to the first 22 patients. Methyl-CCNU (40 mg/m^2) was given orally on Day 1. 5-FU (600 mg/m^2) and TZT (250 mg/m^2) were given iv on Days 1 and 8. The TZT was administered as a 1-hour infusion in 500 ml of 5% dextrose in water. On Days 1 and 8, in addition, ICRF-159 was given orally in three divided doses (total dose of 1 g/m^2 on each day).

Day-8 doses were reduced by 33% for a wbc count of 3000–4000/μl or a platelet count of 75,000–100,000/μl, and they were omitted for blood cell counts below these levels. New cycles were delayed until the wbc count was ≥ 4000 and the platelet count was $\geq 100{,}000$. Patients continued receiving therapy until there was evidence of disease progression. Partial remission was defined as a $> 50\%$ decrease in the sum of the products of the largest diameter of measurable lesions multiplied by the next largest perpendicular diameter. Complete remission was defined as the disappearance of all measurable disease in a patient without signs or symptoms attributable to colorectal cancer. Written informed consent was obtained from every patient.

Twenty-eight patients were entered in the study. Four patients were excluded from further analysis: two because they received inadequate doses of protocol drugs for reasons unrelated to toxicity; one because he had no site of disease which could be evaluated for response; and one because he died from brain metastases after receiving only one dose

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of chemotherapy. Twelve of the remaining 24 patients were female. Five patients were fully active (Eastern Cooperative Oncology Group performance status [PS] 0); ten were ambulatory with symptoms (PS 1); five were bedridden less than half the day (PS 2); and four were bedridden more than half the day (PS 3). Two patients were black and 22 were white. The mean and median age was 65 years, with a range of 41–87. Three patients were in their ninth decade at the time of entry in the study.

Eleven patients had undergone prior curative resection, and 12 patients had undergone prior palliative resection. Six patients had had prior pelvic radiotherapy. At the time of entry in the study, metastases were identified in the liver (13 patients), lung (ten patients), and bone (one patient). Four patients had known disease at the primary tumor site. Information on differentiation of the primary tumor was available for 20 patients: well-differentiated (eight), moderately well-differentiated (nine), and poorly differentiated (three).

RESULTS

Among the 24 patients with adequate trials of therapy, there were only two objective responses: a partial remission for 19 months and a complete remission still ongoing after 6 months. Both responders were female. One had complete regression of inguinal nodes, and the other had substantial regression of multiple pulmonary nodules. The median duration of survival for all 24 patients from time of entry in the study was 5 months, with a range from 2 weeks to > 19 months.

Hematologic toxicity was generally moderately severe, but two patients died as a result of myelosuppression: one with fever and hypotension while leukopenic, and the other from hemorrhage with a platelet count of 40,000/μl. Of the 24 patients, 12 never had a wbc count of < 4000/μl, 15 never had a platelet count of < 100,000/μl, and 14 never became anemic. On the other hand, five patients had a wbc count of 2000–3000/μl and three had a wbc count of 1000–2000/μl. Four patients had a platelet count of 50,000–75,000/μl and two patients had a platelet count of 25,000–50,000/μl.

Nausea and vomiting were reported for nine patients; these were mild in four, moderate in three, and severe in two. No patient had stomatitis, but diarrhea was severe and dose-limiting in a single patient. Two patients had mild infections while on protocol chemotherapy.

Both patients who died as a result of severe myelosuppression did so in the first cycle of treatment. Therefore, test doses of 5-FU (400 mg/m² iv), ICRF-159 (600 mg/m² orally), and TZT (150 mg/m² iv) were added to the protocol to be given 2 weeks before initiation of full-dose chemotherapy. Any patient developing myelosuppression after the test dose was removed from the study. In an effort to escalate doses in the remaining patients, 60 mg/m² of methyl-CCNU was given orally at the start of the first full cycle (2 weeks after the test dose), with 600 mg/m² of 5-FU iv on Days 1, 8, and 15 of the 28-day cycle, 250 mg/m² of TZT on Days 1, 8, and 15, and 1 g/m² of ICRF-159 on Days 1, 8, and 15.

Six patients were treated according to the revised protocol. One had myelosuppression, with a platelet count of 57,000/μl 2 weeks after the test dose, and received no further chemotherapy. Three patients had no toxicity after the test dose, but had considerable hematologic toxicity within the first 2 weeks of full-dose protocol chemotherapy, so that a third dose in the first cycle was not feasible. One of these three patients responded. The other two patients were able to tolerate increased doses without unacceptable myelosuppression, but neither responded.

DISCUSSION

The use of these four agents in combination has no apparent advantage compared to the use of 5-FU alone or 5-FU plus methyl-CCNU. It is possible that different schedules would have more activity, but there is little evidence for schedule specificity for the agents used in the present study or in chemotherapy for advanced colorectal cancer with any agent. It is unlikely, based on our experience with six patients in whom dose escalation was planned, that higher doses of these four drugs would be tolerable for the majority of patients.

The basis for the severe sudden myelosuppression observed in the two patients who died and in the single patient who developed moderate thrombocytopenia after only a single test dose of 5-FU, TZT, and ICRF-159 is unclear. There was no apparent dysfunction of liver or kidneys in these patients. This kind of sudden severe hematologic toxicity was not observed when only TZT and ICRF-159 were administered to a population of patients who had previously received 5-FU (4). Perhaps an interaction of one of these drugs with 5-FU is responsible.

The poor results of the present trial are similar to those recently reported for combinations of methyl-CCNU plus TZT (5), ICRF-159 plus TZT (4), and 5-FU, TZT, and methyl-CCNU (6). Based on these data, it is unlikely that TZT and ICRF-159 will add substantially to the efficacy of combination chemotherapy for advanced colorectal carcinoma.

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cis-Diamminedichloroplatinum(II) Therapy for Advanced Ovarian Cancer[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Eleven of 32 patients (34%) with advanced ovarian cancer previously treated with conventional therapies had partial or complete responses to 5-day courses of *cis*-diamminedichloroplatinum(II) (CDDP) (20 mg/m²/day) with nine showing objective response prior to initiation of the second course. Median duration of response was 6 months (range, 2–18+). Response to CDDP was not correlated with age, pathologic subtype, tumor grade, or bulk disease but was correlated with response to prior treatment. Eight of 15 patients previously responsive to either single alkylating agents or cyclophosphamide plus doxorubicin (Adriamycin) responded to CDDP. In contrast, response to CDDP was observed in only three of 17 patients showing no response to this prior chemotherapy. The highest response rate to CDDP was seen in that subset of patients sensitive to cyclophosphamide and doxorubicin (seven of ten patients).

[Cancer Treat Rep 64:1147–1148, 1980]

Ovarian malignancies now cause one fifth of all cancer deaths among women in the US. Sixty percent of patients present with advanced disease, and approximately 50% of those with apparently localized tumors eventually have disease recurrence (1). Despite the use of extensive surgery, pelvic irradiation, and systemic chemotherapy in recent years, 5-year survival rates have not improved (2). Alkylating agents produce partial response rates of 40%–60% (1) and have been the standard chemotherapeutic approach to disseminated disease. However, recent work (3) indicates that they are more effective when used in combination with other active drugs.

Reports by Wiltshaw and Kroner (4) and Bruckner and co-workers (5) suggest activity of *cis*-diamminedichloroplatinum(II) (CDDP) in ovarian cancer, with response rates of 26.5% and 31.5%, respectively, in studies of 34 and 19 patients. We report the effect of 5-day courses of CDDP on patients with advanced ovarian cancer resistant to chemotherapy with conventional alkylating agents.

METHODS

Patients who had failed conventional therapy and had objectively evaluable and progressive disease were eligible for this study if they had a creatinine clearance of > 30 ml/minute/m², a wbc count of at least 3000/mm³, and a platelet count of > 100,000/mm³. They were treated with CDDP for 5 consecutive days at a dose of 20 mg/m²/day every 4–5 weeks. Prior to treatment, patients were given 1 liter of normal saline. CDDP was added to a 200-ml volume of saline and infused over 60 minutes with mannitol at a dose of 200 mg/m². To be evaluable for response to CDDP, each patient must have received at least one 5-day course. Response was defined as a > 50% reduction in the product of two measurable tumor parameters and/or a fall in CEA (6), when elevated, by ≥ 75% (one patient). Failure was defined as stable or progressive disease. The term complete response is used here to define absence of detectable disease short of laparotomy and/or laparoscopy.

RESULTS

Of the 32 patients treated, eight had partial and three had complete responses (overall response rate, 34%). Nine of the 21 nonresponders had stable disease while receiving CDDP. There were no responses to CDDP in three patients < 40 years old. Five of 12 women aged 40–49 years, four of ten aged

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50–59 years, none of four aged 60–69 years, and two of three aged ≥ 70 years responded. Nine of 11 responders showed objective evidence of disease regression within 1 month of initiation of CDDP therapy. Subjective improvements were often dramatic and correlated well with objective ones. Average duration of response was at least 6 months from start of therapy, with one patient remaining in complete remission at 18 months. There was no clear correlation with tumor grade or pathologic subtype. Average survival from initiation of CDDP therapy was at least 10.7 months for responders versus 6.6 months for nonresponders. None of 11 patients with prior pelvic irradiation responded to CDDP.

Prior treatment status included 12 patients who had received single alkylating agents to which five responded (table 1). Three of the 12 patients subsequently responded to CDDP. Ten of 20 patients responded to prior therapy with cyclophosphamide combined with doxorubicin (Adriamycin). Seven of ten responders to this combination responded to subsequent therapy with CDDP but only one of ten nonresponders.

Eleven of 89 (12%) courses of CDDP which were evaluable for myelosuppression were complicated by leukopenia of < 2000 cells/mm^3, with an average nadir on Day 29. Leukopenia of < 1000 cells/mm^3 was not observed. Thrombocytopenia of < 150,000 or < 50,000 cells/mm^3 was observed in 54 of 82 (66%) and 16 of 82 (18%) courses, with an average nadir on Day 26.

TABLE 1.—Prior treatment status

Response to prior treatment*	Response to CDDP†
Single alkylating agents	
+5/12	+1/5
	−4/5
−7/12	+2/7
	−5/7
Cyclophosphamide and doxorubicin	
+10/20	+7/10
	−3/10
−10/20	+1/10
	−9/10

*Values = No. of patients responding (+) or not responding (−)/No. receiving treatment.

†Values = No. of patients responding (+) or not responding (−) to CDDP/No. of responders or nonresponders to previous treatment.

DISCUSSION

Most chemotherapeutic agents which are active against ovarian cancer are even more effective when used as initial therapy (7). Thus, it is especially noteworthy when a new agent is found to be effective in patients with extensive prior treatment. Such is the case with CDDP. Present data confirm that CDDP is active in ovarian cancer and not fully cross-resistant with conventional alkylating agents. Responders survived an average of 17.5 months from time of diagnosis to initiation of CDDP therapy versus 25.2 months for nonresponders, suggesting that patients responsive to CDDP do not reflect those having intrinsically less aggressive malignancies as judged by the rate with which they exhausted other therapeutic options. The high response rate in patients whose tumors were sensitive to cyclophosphamide plus doxorubicin (seven of ten) suggests that CDDP may be effectively used with these drugs. Since myelosuppression with CDDP was late in onset and never life-threatening, it should be possible to devise clinically tolerable and effective combination chemotherapy programs.

The 34% response rate to our 5-day schedule of CDDP is comparable to those reported by other investigators (4,5) using single-day therapy. Nephrotoxicity and myelosuppression with daily and 5-day regimens appear to be comparable, with less nausea and vomiting with the former. Therefore, while combination chemotherapy programs employing 5-day courses of CDDP have produced dramatic results in testicular cancer (8), the 5-day regimen does not appear to be superior to less intensive regimens in the treatment of ovarian malignancies. Currently available data appear to favor a dose schedule of 50 mg/m^2 of CDDP once every 3–5 weeks.

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Phase II Evaluation of 4′-(9-Acridinylamino)-methanesulfon-*m*-anisidide (AMSA) in Patients With Advanced Colorectal Carcinoma[1,2,3]

Brief Reports and Preliminary Communications

David S. Carroll, Nancy Kemeny,* Garrett Lynch, and Thomas Woodcock[4,5]

4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA) is one of a group of acridine derivatives synthesized by Cain and Atwell (1). The drug exerts an antitumor effect by intercalation with and external binding to DNA (2). Preclinical testing of AMSA demonstrated activity against L1210 implanted murine leukemia as well as against P388 lymphocytic leukemia, B16 melanoma, and spontaneous mammary adenocarcinoma transplanted into mice (1,3,4). A selective activity against hepatic metastases has been postulated for AMSA due to the decreased level of glutathione-*s*-transferase activity in metastatic tissue (3). Although a moderate rise in alkaline phosphatase was seen in seven of 21 patients treated at the National Cancer Institute (Bethesda, MD),[6] most phase I studies have found toxic effects limited to myelosuppression, phlebitis, and occasional nausea and vomiting (5–7). Moderate to severe leukopenia and mild thrombocytopenia were seen when the drug was administered weekly or at 3- or 4-week intervals. Between February and July 1979, a phase II trial of AMSA was conducted in patients with metastatic colorectal carcinoma at the Memorial Sloan-Kettering Cancer Center.

MATERIALS AND METHODS

The initial dose of AMSA was 120 mg/m²; in the absence of leukopenia, subsequent doses were escalated to 150 mg/m². A solution of AMSA in dimethylacetamide was diluted in 250 ml of 5% dextrose in water and administered over 30 minutes. The doses were repeated at 3-week intervals.

Partial remission was defined as a > 50% decrease in the sum of the products of two perpendicular diameters of all measurable disease for at least 1 month. An adequate trial of AMSA was defined as the administration of at least two doses, with or without toxicity.

Twenty-four patients with measurable and histologically confirmed advanced colorectal carcinoma were entered in the study. The median age was 56 years (range, 37–71), and the median Karnofsky performance status was 80 (range, 60–90). The drug was given to 15 men and nine women. The sites of metastatic disease were: liver, 17 (83%); lung, 14 (48%); pelvic mass, six (25%); and vulva, one (4%). The median time from diagnosis of metastatic disease to entry in this study was 14 months (range, 5–42). Nine patients had received prior radiotherapy and all had received prior chemotherapy. The previous chemotherapy included methyl-CCNU and 5-FU in 22 patients (92%), *N*-(phosphonacetyl)-L-aspartic acid (PALA) in 11 patients (46%), and vindesine in eight patients (33%).

RESULTS

Twenty-two of the 24 patients entered had an adequate trial. Of the two patients who had inadequate trials, one died from progressive disease after only one dose, and the other died of sepsis and leukopenia 6 days after the first dose. The median total dose of AMSA for the evaluable patients was 330 mg/m² (range, 210–840) and the median number of doses was three (range, two to seven). Each patient had measurable progressive disease within 3 weeks after the last dose. No tumor regression was noted during any course of treatment.

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[3]AMSA was supplied by Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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[5]We wish to acknowledge the technical assistance of Jane Howard and the secretarial assistance of Cathie Cassidy.

[6]Minutes of the Phase I Working Group Meeting. Investigational Drug Branch, Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD, May 15, 1978.

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Leukopenia was the most frequent toxic effect seen in this study. The median wbc count was 2900/mm³ (range, 400–5800) and the median duration from the administration of the drug to the wbc count nadir was 17 days (range, 6–19). Five patients (23%) had wbc counts of ≤ 2000/mm³. Six patients (27%) had platelet counts of < 150,000/mm³, and only two of these patients had platelet counts of < 100,000/mm³. Thrombocytopenia was also seen approximately 2 weeks after the dose of AMSA. Recovery from blood cell count depression was rapid, and in no case was the interval between doses increased to > 3 weeks. Two patients tolerated dose escalation of 150 mg/m² and two required dose reduction to 90 mg/m².

Myelosuppression was not related to initial liver function tests. Bilirubin was normal in all patients at the initiation of therapy. Only two of the eight patients with an alkaline phosphatase of < 300 units/liter (normal, 40–82) had myelosuppression, while six patients with an alkaline phosphatase of > 300 units/liter had myelosuppression.

DISCUSSION

Twenty-four patients with advanced colorectal carcinoma were treated with AMSA at 3-week intervals. No tumor regression was seen. Leukopenia and thrombocytopenia were the observed toxic effects, and leukopenia contributed to fatal sepsis in one patient with mild ascites but normal creatinine and liver function tests. No nonmyelosuppressive toxicity was seen in this trial. Deterioration of liver function tests occurred in several cases, but these abnormalities were always related to an increase in metastatic hepatic disease documented by liver scan and measurements. It can be concluded that AMSA, when given at this dose and schedule to previously treated patients with advanced colorectal carcinoma, is not an effective drug.

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Effects of Chemotherapy With Mitomycin C and 5-FU on Pancreatic Functions in Patients With Gastric Cancer[1]

Brief Reports and Preliminary Communications

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Several chemotherapeutic agents have been associated with pancreatic toxicity or carbohydrate abnormalities, including alloxan (1,2), cyclophosphamide (3), tubercidin (2), streptozotocin (2,4,5), L-asparaginase (6,7), and busulfan (8). Recently, mithramycin has also been reported to have an effect on pancreatic beta cell function (9). However, the effects of chemotherapy with mitomycin C and 5-FU on pancreatic functions have not been studied systematically in patients and experimental animals. I have therefore examined both endocrine and exocrine functions of the pancreas in patients who were treated with these two agents.

MATERIALS AND METHODS

Eighteen patients with gastric cancer were studied. No patient had clinical evidence of metastasis, metabolic or endocrine disease, or conditions known to affect glucose tolerance, and all patients were in fairly good and stable condition without discernible impairment of liver and kidney function during the study period. The initial course of combination chemotherapy given to these patients consisted of 0.4 mg/kg of mitomycin C iv on Day 1 and 10 mg/kg of 5-FU iv daily starting on Day 1 for 5 consecutive days. No other therapeutic agents were added or omitted during the observation period. For studies of endocrine function, blood samples were collected from fasting patients before and at 30, 60, 90, 120, and 180 minutes after oral administration of 50 g of glucose. Blood glucose was determined by the glucose oxidase method (10). Plasma immunoreactive insulin was determined by the double antibody method (11). C-peptide immunoreactivity was measured by an immunoassay with rabbit anti-C-peptide antiserum prepared against synthetic human C-peptide (12). For exocrine function examination, urine samples were collected during 6 hours after oral administration of *N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid, and p-aminobenzoic acid was measured by the method of Imondi et al (13). These tests were conducted immediately before chemotherapy and between Days 5 and 7 after termination of chemotherapy. Statistical analysis was performed by the t test for paired values.

RESULTS AND DISCUSSION

Fasting blood glucose and insulin levels were unchanged after chemotherapy (table 1). Compared with the values before chemotherapy, there were significant changes after chemotherapy in the values in response to ingestion of 50 g of glucose: blood glucose concentration was significantly greater at 30, 60, 90, and 180 minutes, while immunoreactive insulin and C-peptide levels were significantly lower at 30, 60, and 90 minutes. In contrast, the amount of *p*-aminobenzoic acid recovered in the urine during the 6 hours was unchanged after chemotherapy.

The results indicate that chemotherapy with mitomycin C and 5-FU reduced pancreatic beta cell secretory response to hyperglycemic stimulus. Because many patients are currently receiving both mitomycin C and 5-FU in large cumulative doses in a multicenter gastric cancer chemotherapy trial, it is important to clarify this association, and I am undertaking microscopic studies using the rat model. Physicians should be aware of the possible association between reduced pancreatic beta cell function and this cytotoxic regimen.

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TABLE 1.—Effects of mitomycin C and 5-FU therapy on pancreatic functions in patients with gastric cancer

Function test*	No. of patients	Values† before glucose administration	Response† to oral administration of 50 g of glucose at—				
			30 mins	60 mins	90 mins	120 mins	180 mins
Blood glucose (mg/dl)							
Before chemotherapy	18	97.8 ± 1.9	153.2 ± 6.0	173.0 ± 6.6	161.2 ± 7.7	134.0 ± 7.4	104.4 ± 5.7
After chemotherapy	18	100.4 ± 2.0	176.6 ± 6.7	193.0 ± 7.1	180.2 ± 6.6	137.7 ± 7.5	119.3 ± 5.6
P value		NS	< 0.0025	< 0.002	< 0.005	NS	< 0.001
Immunoreactive insulin (microunits/ml)							
Before chemotherapy	18	10.6 ± 2.2	78.6 ± 13.2	85.3 ± 11.7	73.3 ± 12.3	43.2 ± 6.2	21.4 ± 4.8
After chemotherapy	18	10.1 ± 1.6	48.2 ± 6.9	55.0 ± 8.6	45.8 ± 9.0	36.1 ± 6.8	22.3 ± 4.0
P value		NS	< 0.01	< 0.01	<0.025	NS	NS
C-peptide (ng/ml)							
Before chemotherapy	8	1.34 ± 0.06	8.45 ± 0.46	9.63 ± 0.37	8.03 ± 0.35	6.21 ± 0.26	3.90 ± 0.24
After chemotherapy	8	1.44 ± 0.08	6.31 ± 0.55	8.05 ± 0.60	6.71 ± 0.16	5.89 ± 0.27	4.06 ± 0.18
P value		NS	< 0.01	< 0.05	< 0.05	NS	NS
Cumulative 6-hr excretion of *p*-aminobenzoic acid (%) (14 patients)							
Before chemotherapy		86.4 ± 2.6					
After chemotherapy		84.0 ± 3.0					
P value		NS					

*Tests were conducted immediately before chemotherapy and between Days 5 and 7 after termination of chemotherapy. Statistical analysis was performed by the t test for paired values.

†Mean ± SE. NS = not significant.

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Phase I Clinical Trial of Weekly Iv 2,3-Dihydro-1*H*-imidazo[1,2-*b*]pyrazole (IMPY)[1,2]

Brief Reports and Preliminary Communications

Charles L. Vogel,* John M. Denefrio, darLene C. Padgett, and Michael A. Silverman[3,4]

SUMMARY

In a phase I trial, 2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole was administered iv weekly at doses ranging from 0.1 to 3.0 g/m²/wk to patients with refractory metastatic solid tumors. Although gastrointestinal toxicity was tolerable and neither granulocyte nor platelet toxicity was seen, significant dose-limiting hemolysis was encountered at doses of 1.5 g/m²/wk. While no definite tumor regressions were seen in the 32 patients evaluable for response, two patients (squamous cell carcinoma of lung and tonsil) had clinically useful improvement.

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2,3-Dihydro-1*H*-imidazo[1,2-*b*]pyrazole (IMPY) is a ribonucleotide reductase inhibitor selected for clinical trial because of activity against L1210 leukemia resistant to the structurally related dimethyl (DTIC) and bis-chloroethyl (BIC) imidazole carboxamide compounds; the ribonucleotide reductase inhibitors, thiosemicarbazone and guanazole; and the S-phase-specific, DNA polymerase inhibitor, cytosine arabinoside. However, L1210 leukemia resistant to another ribonucleotide reductase inhibitor, hydroxyurea, was also resistant to IMPY (I. Kline, personal communication). There was a relative lack of schedule dependency in preclinical studies which is at least partially explained on the basis of in vivo pharmacokinetics (1) related to extensive conversion of the parent drug to active derivatives, such as the β-ribosyl compound (2). The weekly schedule was chosen in the present trial since a similar schedule in large animal toxicology studies permitted administration of larger total doses with less toxicity than other schedules tested. Hemolysis was dose-limiting in large animal toxicology studies where the monkey was more sensitive than the dog. Hepatotoxicity and some renal impairment were also noted at high doses.

METHODS

Pretreatment and weekly laboratory evaluation included cbc, platelet and reticulocyte counts, SMA-17, serum amylase, direct and indirect Coombs' tests, haptoglobin, and urinalysis. Additional studies done in patients at the highest dose levels included serum iron and iron-binding capacity, serum folate and B_{12} levels, stools for occult blood, and urine hemosiderin. Most patients had a hemoglobin concentration of > 10 g/100 ml, normal wbc and platelet counts, and normal serum creatinine and bilirubin levels prior to entry in the study.

At doses up to 1.5 g/m² IMPY was diluted in distilled water for injection and given by iv push over 5–10 minutes. At higher doses the drug was reconstituted in water for injection, diluted to 250 ml in 5% dextrose in water, and administered over 30–60 minutes. Doses were given weekly for 4 consecutive weeks followed by a rest period of 2 weeks prior to initiation of a second course of therapy. The starting dose of 0.1 g/m²/wk was extrapolated from the toxic dose low in a 5-day dose schedule in the monkey (0.5 g/m²/day × 5). Less than one third of the toxic dose low was chosen as the starting dose because of the lack of comparability of schedules (weekly in clinical trial; 5-day course in the monkey). Dose escalations were planned using a modified Fibonacci scheme. Routine pre-medication with antiemetics was not done unless the first drug dose caused significant nausea or vomiting. All patients had measurable or evaluable tumor for assessment of antitumor response.

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[3]Divisions of Breast Cancer and Medical Oncology, Department of Oncology, University of Miami School of Medicine, and the Comprehensive Cancer Center for the State of Florida.

[4]We are indebted to Mohan S. Khurana, Daniel D. Nixon, R. Judith Ratzan, Peter W. A. Mansell, and Michael B. Troner who participated in the clinical care of some of the study patients.

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RESULTS

Thirty-seven patients with metastatic solid tumors refractory to standard therapeutic regimens received a total of 41 trials of weekly IMPY. Each of four patients had evaluable trials at two different dose levels of ≤ 0.67 g/m²/wk; in each there was a 1-month rest period after the fourth dose of the preceding course. Thirty-two of 37 patients (36 of 41 trials) were considered fully or partially evaluable for toxicity and/or antitumor response (table 1). Of the five unevaluable patients, three had a low performance status and died from advancing disease and two became noncompliant after only one drug dose.

In 18 evaluable trials at doses ranging from 0.1 to 1.0 g/m²/wk there was no significant gastrointestinal or hematologic toxicity, although one patient with colon cancer, liver metastases, and an alkaline phosphatase of 1100 IU treated at 0.67 g/m²/wk had a hemoglobin decrease of 3 g/100 ml. Even at higher doses CNS and cardiovascular toxic effects were not encountered and there was no clinically significant gastrointestinal bleeding. Anorexia and tolerable nausea were seen at doses of > 1 g/m², but in no patient was vomiting severe enough to cause termination of therapy. Patients complained of lethargy at doses of > 1.5 g/m²/wk, but, in most cases, this could be explained by anemia or disease progression. No patient had a clinically significant fall in wbc count and only two patients had a fall in platelet count. In one of the latter patients, treated at 2.25 g/m²/wk, prior treatment with nitrosoureas could have contributed to a fall in the platelet count from 128,000 to 85,000/mm³. The other patient had a precipitous fall in platelet count during a hemolytic transfusion reaction on the day of his fourth dose of IMPY. Post-mortem examination, however, revealed the cause of death to be massive intra-abdominal tumor involvement with perforation of the colon.

Drug-induced Coombs'-negative hemolysis was the dose-limiting toxic effect (table 2). At a dose of 3 g/m²/wk seven of eight patients required transfusion. Hemolysis was seen at doses of 1.5 and 2.25 g/m²; there was a tendency for this to be more severe in patients with pre-existing elevations of the serum alkaline phosphatase. Hemolysis generally occurred with the first drug dose, with acute elevations of the SGOT and reticulocyte count. Only

TABLE 1.—Patient characteristics

Characteristic	Total	No. of patients: Dose levels (g/m²/wk) 0.1	0.2	0.33	0.5	0.67	1.0	1.5	2.25	3.0
Evaluable	32									
Males:females (mean ages)	23:9 (61:56)									
Karnofsky performance status										
> 80%	19									
50–79%	12									
< 50%	1									
Type of cancer										
Melanoma		2	0	1*	0	1	0	0	0	3
Colon		0	0	0	0	1	1	1	2	1
Breast		0	0	0	0	0	1	1	1	0
Sarcoma		0	1	0	0	1*	0	1	0	1
Sweat gland		0	0	0	0	0	0	1	0	0
Head and neck		1	0	0	0	0	0	0	1†	1†
Unknown primary										
Squamous cell carcinoma		0	0	0	1	0	0	0	0	0
Adenocarcinoma		0	1	0	0	0	0	0	0	0
Lung										
Oat cell carcinoma		0	0	0	0	0	1	0	0	1
Large cell carcinoma		0	0	0	1	0	0	0	0	0
Squamous cell carcinoma		0	0	1	0	1*	0	0	2‡	1
Adenocarcinoma		0	1	0	1*	0	0	0	0	0
Totals		3	3	2	3	4	3	4	6	8

*Treated at 2 dose levels.
†Same patient.
‡1 of these patients de-escalated from 3.0 g/m².

TABLE 2.—Toxicity of weekly IMPY

Dose ($g/m^2/wk$)	No. of trials	Emesis	Hemoglobin decrease			Mean peak % reticulocytes (range)	Transfused	Mean SGOT increase in IU (range)
			< 3 g/100 ml	> 3 g/100 ml	Mean in g/100 ml (range)			
1.5	4	3	1	3	3.0 (1.4–4.2)	3.7 (2.2–5.4)	1	17 (0–41)
2.25	6	3	3	3	3.7 (0.8–5.3)	6.0 (1.2–15.2)	1	80 (0–309)
3.0	8	2	2	6	4.4 (2.0–6.1)	7.7 (5.1–9.6)	7	72 (48–110)

one patient in the entire study had a low serum haptoglobin level. This occurred on the fourth day after her first drug dose at 2.25 g/m^2 with recovery on the day of her second dose. This same patient, studied daily for 4 days, also had a reticulocyte count of 15.2% and an SGOT which rose from 19 to 328 IU with gradual decrease to normal over the ensuing month. More frequent testing as done in this patient would likely have revealed more transient abnormalities of haptoglobin, reticulocyte count, and SGOT in other patients. Possible hepatotoxicity was seen in one patient treated at a dose of 3 g/m^2 who had a hemoglobin decrease of 1.9 g/100 ml, liver enlargement, and reversible increases in bilirubin and SGOT 5 days after one dose of IMPY. A concomitant increase in alkaline phosphatase did not decline over a 1-month period prior to his death from progressive tumor. While an occasional patient treated at doses of < 1.0 $g/m^2/wk$ had transient, slight SGOT elevations, all had increases at 3 g/m^2. Only three of six patients treated at 2.25 g/m^2 had SGOT increases, but this was dramatic in one patient leading to a high mean value.

Only two patients treated at doses of > 1.0 g/m^2 received more than one course of IMPY. One patient with an ulcerating, unresectable squamous cell head and neck cancer received two courses at 3 g/m^2 and one course at 2.25 g/m^2 which rendered the tumor suitable for palliative resection. Although multiply transfused at the 3-g/m^2 dose, he was not transfused at the 2.25-g/m^2 dose. A second patient with squamous cell lung cancer had subjective improvement, a 16-pound weight gain, and a period of disease control lasting for 6½ months. During this time he required a total of 17 units of packed rbcs when receiving a dose of 3 g/m^2 and an additional 10 units while receiving 2.25 g/m^2.

DISCUSSION

In this phase I clinical trial of weekly iv IMPY the dose-limiting toxic effect was hemolysis. IMPY has been shown to generate hydrogen peroxide in rbcs in vitro and to be associated with methemoglobin formation in vivo, although the precise mechanism of hemolysis remains uncertain (3). Neurotoxicity and cardiotoxicity were not seen, bone marrow suppression was not encountered, and gastrointestinal toxicity was tolerable. There was no clinically significant gastrointestinal blood loss, a possible complication suggested by discovery of high concentrations of the drug in gastric juice (3). One patient had deranged liver function tests after one dose of IMPY, suggesting hepatotoxicity. Although no patient in this phase I study had unequivocal evidence of tumor regression, two patients, both with squamous cell cancers, had clinically useful subjective improvement. One of these patients, whose disease was previously deemed unresectable, was subsequently able to undergo palliative resection of a recurrent cancer involving the mandible.

The hint of clinical activity in squamous cell cancer, the drug's ability to synchronize human lymphoid cells (4) and L1210 and P388 ascites (5) in vitro, and the relative lack of myelosuppression in this and other phase I clinical trials would appear to make the drug interesting enough to advance to phase II clinical trials. On the other hand, extreme dose-limiting nausea, vomiting, and fatigue noted by one investigator at 1200 $mg/m^2/day \times 5$ (C. Haas, personal communication) and hemolysis seen in this study using a weekly schedule and by Neidhart using three additional drug schedules (single bolus, daily × 5 bolus, and 5-day continuous infusion) have tempered enthusiasm for expanded clinical trials. Neidhart feels that nontoxic dose levels should still be effective for inhibition of DNA synthesis. Since the weekly schedule was better tolerated in animal toxicology studies and produced little subjective toxicity at doses of ≤ 1.5 $g/m^2/wk$ in the present clinical trials, limited trials of this dose and schedule of IMPY in squamous cell cancers would be of interest before abandoning the drug because of undue toxicity.

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Phase II Trial of 4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA) in the Treatment of Advanced Non-Hodgkin's Lymphoma[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

A disease-oriented trial of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide) given once every 3 weeks was undertaken in patients with advanced non-Hodgkin's lymphoma. None of 15 adequately treated patients achieved complete or partial remissions. Toxicity was mild-to-moderate, consisting of myelosuppression (principally granulocytopenia) and malaise. The lack of any major responses in this number of patients suggests that, in this schedule, the drug is not useful in therapy for advanced non-Hodgkin's lymphoma.

[Cancer Treat Rep 64:1157–1158, 1980]

4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA), an acridine dye derivative demonstrating substantial preclinical antitumor activity (1), is currently undergoing disease-oriented trials at several institutions. The compound appears to act by intercalation with DNA (2). In initial clinical studies at this institution (3) and elsewhere (4,5), myelosuppression (principally granulocytopenia) was found to be the dose-limiting toxic effect. Encouraged by an early report showing a complete remission in a patient with nodular lymphoma treated with AMSA (4), we initiated a phase II trial using this drug in a similar schedule in patients with advanced non-Hodgkin's lymphoma.

MATERIALS AND METHODS

Between April and October 1979, 21 patients with histologically confirmed diagnoses of non-Hodgkin's lymphoma were entered in this study; entry requirements included the presence of objectively measurable disease and a performance status of ≥ 50 (Karnofsky). All patients had initially adequate hematologic status (wbc count $> 4000/mm^3$ and platelet count $> 120{,}000/mm^3$) and had not received other chemotherapy or radiation therapy for a minimum of 4 weeks prior to entry. Signed informed consent was obtained from each individual.

Before starting therapy, all patients had a complete history and physical examination and underwent additional procedures as needed to define the extent of disease. Pretreatment laboratory tests included cbc, platelet count, liver function tests (SGOT, alkaline phosphatase, lactate dehydrogenase, and total bilirubin), serum creatinine, and chest roentgenogram. While the patients were receiving therapy, a physical examination, cbc, and platelet count were performed every 3 weeks; an additional blood cell count was obtained 7–14 days after each dose of AMSA.

An adequate trial required a minimum of two doses of AMSA given 3 weeks apart which produced hematologic toxicity (wbc count $< 3000/mm^3$ or platelet count $< 100{,}000/mm^3$). Antitumor effects were assessed as conventionally defined (5).

The initial dose of AMSA (supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD) was 120 mg/m² with escalation of the dose by 30 mg/m² every 3 weeks in the absence of hematologic toxicity; in patients who had received pelvic irradiation, the initial dose was attenuated to 90 mg/m² with the same escalation schedule. The prescribed dose of AMSA was added to 250 ml of a 5% dextrose solution and infused over 30 minutes.

RESULTS

Twenty-one patients were entered in this study; 15 were considered adequately treated and evalu-

[1]Received Dec 18, 1979; revised Feb 29, 1980; accepted Mar 12, 1980.

[2]Supported in part by contract N01-CM-57043 (Division of Cancer Treatment) and Public Health Service grants CA-09207 (National Research Service Award; *R. P. Warrell*) and CA-05826 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Developmental Chemotherapy (*R. P. Warrell* and *C. W. Young*) and Hematology/Lymphoma (*D. J. Straus*) Services, Memorial Sloan-Kettering Cancer Center, New York, NY.

[4]We thank Jane Howard for assistance with data collection.

**Reprint requests to:* Dr. R. P. Warrell, Jr, Developmental Chemotherapy Service, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

able for response. The histologic diagnoses and pretreatment characteristics are presented in table 1. These patients received a median dose of 120 mg/m² (mean, 130 mg/m²; range, 90–210 mg/m²); a median of two doses (range, two to four) per patient were administered.

There were no complete or partial remissions in any of these 15 patients. Minor regressions of disease were seen in two patients, one of whom had 50% shrinkage of his tumor which lasted only 2 weeks and progressed despite further therapy at an escalated dose. Six patients were classed as inadequately treated. On review, four of these patients were believed to have had lower than acceptable performance status for entry (30–40) and received only a single dose of AMSA with no response. One patient was removed from the study by his attending physician because of rapid disease progression after one dose; another patient required emergency radiotherapy for superior vena caval compression 7 days after receiving his second dose of AMSA but prior to experiencing hematologic toxic effects.

Toxicity

In general, AMSA was well-tolerated. As in previous studies the major toxic effect was myelosuppression, with leukopenia being more prominent than thrombocytopenia; nine of 15 patients had a wbc count nadir of < 2500/mm³ while only three had a platelet count nadir of < 100,000/mm³. Anemia (decrease in hemoglobin concentration of > 2.5 g/100 ml) was noted in three patients and improved after the drug was discontinued. Most patients who received more than two doses of AMSA complained of generalized weakness, anorexia, and malaise which persisted up to 2 weeks after the previous dose. Other toxic reactions were uncommon, being limited to nausea and vomiting (two patients) and urticaria at the infusion site without other evidence of hypersensitivity (one).

TABLE 1.—Characteristics of evaluable patients treated with AMSA

Characteristic	No. of patients
Total evaluable	15
Performance status (Karnofsky)	
70–80	10
50–60	5
Histologic diagnosis (Rappaport)	
Diffuse histiocytic	6
Diffuse mixed histiocytic/lymphocytic	2
Diffuse poorly differentiated lymphocytic	3
Nodular poorly differentiated lymphocytic	4
Prior radiotherapy	9
Median duration of disease in yrs (range): 3 (0.5–13)	
Median No. of previous chemotherapy regimens (range): 2 (1–4)	
Median age in yrs (range): 51 (32–72)	

DISCUSSION

The lack of response to AMSA in this study was somewhat surprising in view of the initially favorable case report (4). Despite the fact that patients in this study were heavily pretreated, they were all of outpatient performance status and several have subsequently responded to other investigational agents; moreover, a quite stringent definition of "adequate treatment" was applied to avoid missing potentially useful responses. Since this drug is rapidly excreted, we do not exclude the possibility that improved response rates might be seen using an alternative dose schedule; however, the schedule of every 3 weeks employed for this study appeared superior to other schedules investigated in phase I trials, given the 10–14-day delay in myelosuppression.

Because of the absence of any major responses in these 15 patients, we conclude that AMSA is likely to have a true major response rate of < 20% ($P < 0.05$) (6) and, as used in this schedule, has limited therapeutic utility in advanced non-Hodgkin's lymphoma.

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LETTERS

Coronary Artery Disease After Treatment With Bleomycin and Vinblastine[1]

A recent report (1) described two patients, ages 20 and 25 with testicular cancer, who developed severe coronary artery disease (CAD) following chemotherapy with *cis*-diamminedichloroplatinum(II) (CDDP), vinblastine, and bleomycin. It was suggested that CDDP may act as a potentiating agent in the development of atherosclerosis. Two of our patients (table 1) with metastatic testicular cancer developed CAD following therapy with vinblastine and bleomycin; they did not receive CDDP.

TABLE 1.—Characteristics of patients with CAD

Characteristic	Patient No. 1	Patient No. 2
Age	40	45
Risk factors		
Smoking	+	+
Hypertension	–	–
Hyperlipidemia	–	–
Family history	–	–
Chemotherapy	Actinomycin D, vinblastine, bleomycin	Actinomycin D, vinblastine, cyclophosphamide, methotrexate, bleomycin, mithramycin

CASE REPORTS

Patient 1

Patient 1 is a 38-year-old male who had an inguinal orchiectomy for an embryonal cell carcinoma. A retroperitoneal lymph node dissection also revealed embryonal cell carcinoma. He was given 3 mg of actinomycin D, 270 units of bleomycin, and 51 mg of vinblastine during four courses of adjuvant chemotherapy. Maintenance therapy with vinblastine was given for 15 months. Twenty-seven months after orchiectomy, an inferior myocardial infarction occurred. Postinfarction angina developed. A coronary angiogram demonstrated complete occlusion of the circumflex artery and 30% narrowing of the left coronary artery. He is disease-free 57 months after orchiectomy and has developed Raynaud's phenomenon this past year.

Patient 2

Patient 2 is a 40-year-old male who had an inguinal orchiectomy for a seminoma plus embryonal cell carcinoma of the testes. A retroperitoneal lymph node dissection revealed embryonal cell carcinoma. He was given adjuvant actinomycin D, methotrexate, and cyclophosphamide for 10 months. Hepatic metastases, confirmed by laparotomy, occurred and he received 720 units of bleomycin, 125 mg of vinblastine, and mithramycin over a 13-month period. After completion of chemotherapy, a laparotomy revealed multiple necrotic hepatic nodules. Maintenance vinblastine was given for 2 years during which time he noted Raynaud's phenomenon. Fifty-four months after orchiectomy, he developed anterior chest pain and dyspnea. An electrocardiogram revealed a new inferior myocardial infarction, and a chest roentgenogram and echocardiogram disclosed a pericardial effusion. A pericardiocentesis was negative for malignancy, and tumor markers were within normal limits. Coronary angiography was not performed. He is currently disease-free 76 months after orchiectomy.

COMMENT

These two patients represent examples of CAD occurring after treatment with bleomycin and vinblastine. Although both of our patients had a history of smoking, and were older than the patients reported by Edwards et al (1), the association between the prior chemotherapy and the development of CAD cannot be overlooked.

A possible explanation for the accelerated atherosclerosis seen in our patients and in those reported by Edwards et al is the bleomycin and vinblastine combination. This combination has been linked to the development of Raynaud's phenomenon (2–6). Thirteen (34%) of the last 38 patients with testicular tumor treated at the University of Minnesota with vinblastine, bleomycin, and CDDP

[1]Supported in part by Public Health Service grant CA-19527 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services; the American Cancer Society Regular Clinical Fellowship CF 4308A (N. J. Vogelzang); the Whirlpool Foundation; the Minnesota Medical Foundation; and the Masonic Memorial Hospital Fund, Inc.

have clinical evidence of Raynaud's phenomenon (7). The example of CAD plus Raynaud's phenomenon as seen in both patients may indicate widespread arterial damage by the bleomycin and vinblastine combination. The relative contribution of CDDP to these arterial lesions is unknown. Several hypotheses have been advanced to account for the phenomenon (2–6), but currently no data are available to support the theories.

A second consideration in these patients is that the CAD is associated with actinomycin D. Both cases reported by Edwards et al (1) and both of our patients received actinomycin D. However, actinomycin D has not been associated with the late development of CAD.

In summary, the bleomycin and vinblastine with or without CDDP combination may be a cause of Raynaud's phenomenon and premature CAD. Such events need further documentation in view of the significant roles these agents play in the cure of testicular cancer.

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Melphalan in Metastatic Cancer of the Prostate

In reviewing the 1979 literature on alkylating agents for Cancer Chemotherapy Annuals, my attention has been drawn to the letter by C. R. Franks in the February issue of *Cancer Treatment Reports* (63:228–229, 1979) in which he describes a pilot study of melphalan in metastatic cancer of the prostate. The basis for using melphalan is a quoted literature report that melphalan circulates in the blood as the nontoxic glucuronide that may be reactivated in prostate tissue which is high in glucuronidase. To my knowledge, there is no evidence that melphalan is converted to a glucuronide in vivo, and recent investigations would indicate that this metabolite is not formed (1,2). Dr. Franks' source is a review (3) which in turn quotes an earlier paper (4). From this earlier paper one only obtains evidence that melphalan (in combination with mitomycin C and vincristine) is, in fact, not active against prostate cancer. However, also in the paper is a statement that aniline mustard is a useful agent in the treatment of prostate cancer because of its known metabolism to the glucuronide (5) which may be activated by prostate β-glucuronidase to *p*-hydroxyaniline mustard. Could the author possibly have confused L-phenylalanine mustard (sometimes referred to as L-PAM) with aniline mustard (sometimes referred to as AM)?

The two chemicals, of course, differ quite considerably in their chemical, biochemical, pharmacologic, antitumor, and toxicologic properties.

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Lactate Dehydrogenase Virus Infection of Murine Tumors: No Effect on Response to Chemotherapy[1]

Lactate dehydrogenase virus (LDV), first described by Riley et al in 1960 (1), is recognized as a common contaminant of many experimental murine tumors. It appears to replicate only in murine macrophages, has been shown to interfere with a number of immune functions, but is not associated with any gross pathology (2,3). However, contradictory reports have appeared as to the influence of LDV infection on tumor growth (2,4,5), and no data have yet been presented regarding possible effects on the results of experimental chemotherapy.

During the course of our therapeutic experiments using the Ridgway osteogenic sarcoma (ROS), it became clear that tumor-bearing AKR mice were infected with LDV, since an 8.5-fold rise in plasma LDH activity was detected by standard spectrophotometric assay following tumor passage (table 1). It then became necessary to derive an LDV-free ROS subline, and we found that this could conveniently be achieved by tumor passage through tissue culture, as described by Plagemann and Swim (4).

Whole tumor fragments were freed from hemorrhagic and necrotic debris, and were digested at room temperature for 10 minutes using an enzyme solution containing 0.1% trypsin, 0.1% collagenase, and a trace of DNAase. Cells were thoroughly washed and cultured in RPMI-1640 nutrient medium supplemented by L-glutamine, 15% fetal calf serum, and 25 mM Hepes buffer; under these conditions, ROS cells consistently grew as discrete colonies. These were passaged three times by trypsinization (0.1% trypsin in phosphate-buffered saline); by this means complete separation of tumor cells from LDV-infected host macrophages was achieved. After 4–6 weeks in tissue culture, tumor cell colonies were pooled and directly inoculated through subcutaneous incisions in the flanks of recipient female AKR mice. Tumors became palpable 3–4 weeks later, and the absence of LDV infection in the mice was confirmed by plasma LDH estimation (table 1). Growth rates over 10 days, assessed by caliper measurements, were compared in the virus-infected and virus-free tumor-bearing animals (six per group). No significant difference in the overall mass doubling time (3.1 days in each group) was seen, nor was any difference noted in the take rate (90%–95%) at subsequent passages.

Groups of LDV-infected and LDV-free tumor-bearing mice (six per group) were treated with a single iv dose of actinomycin D (500 μg/kg) and tumor changes were measured 10 days after treatment. No significant difference in response was seen as a result of LDV infection (table 2). In addition, as part of a separate study, an actinomycin D-resistant subline of the ROS which had been derived from the original tumor by repeated treatment in vivo underwent a similar process of in vitro passage to obtain an LDV-free, drug-resistant tumor. Following this process, the ROS subline retained its resistance to actinomycin D, and no significant difference was apparent either in growth rate or in tumor response to actinomycin D with respect to the presence of LDV infection (table 2).

TABLE 1.—Effect of in vitro tumor passage on LDH levels in ROS-bearing mice

Mice (No.)	Mean LDH level in IU/liter (SE)
Normal (6)	385 (82)
Tumor-bearing before in vitro passage (12)	3278 (350)
Tumor-bearing after in vitro passage (13)	419 (38)

TABLE 2.—Influence of LDV infection on chemotherapeutic response of ROS tumors that are sensitive and resistant to actinomycin D at a dose of 500 μg/kg iv

Time	Mean tumor weight in g (SE)			
	LDV-contaminated mice		LDV-free mice	
	Treated	Control	Treated	Control
	Sensitive ROS			
Day of treatment	1.61 (0.19)	1.23 (0.12)	1.40 (0.25)	1.20 (0.30)
10 days after treatment	0.19 (0.05)	11.80 (1.0)	0.32 (0.08)	11.53 (1.21)
	Resistant ROS			
Day of treatment	1.27 (0.22)	1.28 (0.20)	1.43 (0.21)	1.23 (0.12)
10 days after treatment	5.39 (0.48)	11.70 (1.26)	6.75 (1.38)	12.22 (0.62)

[1]Supported by the Cancer Research Campaign.

These data therefore suggest that despite the marked effect which can be observed particularly on the reticuloendothelial system of the host as a result of LDV infection, its presence does not appear to influence the response of murine tumors to chemotherapy.

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March 10, 1980

Cellulitis and Fibrosis Due to *cis*-Diamminedichloroplatinum(II) (Platinol) Infiltration

The gastrointestinal, otologic, and renal toxicity of *cis*-diamminedichloroplatinum(II) (CDDP) is well-known. We recently saw a patient who developed moderately severe cellulitis and fibrosis following an extravasation of CDDP.

CASE REPORT

A 66-year-old white male was referred to the Section of Medical Oncology with the diagnosis of transitional cell carcinoma of the bladder metastatic to the lung. He was entered in an Eastern Cooperative Oncology Group protocol on which he was to receive CDDP, cyclophosphamide, and doxorubicin.

He received the doxorubicin and cyclophosphamide through a freely running iv line in the left forearm without difficulty. For purposes of hydration it was felt that a new iv should be started in the right forearm. After receiving 1 liter of fluid, furosemide, and mannitol, the CDDP infusion was begun. One hundred and five milligrams of CDDP was dissolved in 250 ml of 5% dextrose in one-half normal saline. Halfway through the infusion, the patient walked to the restroom and, when he returned, a large infiltration was noted. The area of swelling measured 7 × 6 × 2 cm. Warm soaks were applied to the area over the next 24 hours.

Two weeks later a moderately severe cellulitis had developed at the site of extravasation with a tender, indurated area measuring 8 × 7 cm, and erythema extending 12 cm proximally, suggesting perivascular inflammation. Over the next 4 weeks the erythema subsided and the indurated area shrank to 5 cm in diameter. At this time the area was hard and only minimally tender. By Week 10 there was only a small amount of residual fibrosis and no limitation of movement. During this period the patient received two additional courses of all three drugs without incident.

COMMENT

Several chemotherapeutic agents (eg, doxorubicin and mechlorethamine) are known to cause severe cellulitis, often resulting in fibrosis if extravasation occurs. We believe this is the first report of cellulitis following the extravasation of CDDP. Dermatologic reactions to platinum have been described. Workers exposed to platinum salts may develop an illness (platinosis) characterized by cutaneous (erythema, pruritis, and urticaria) and pulmonary (dyspnea and asthma) manifestations (1). However, this is felt to be an allergic reaction and cellulitis has not been described.

Fortunately, in this case, the residual fibrosis was minimal. Nevertheless, the prolonged cellulitis illustrates the need to exercise caution when infusing CDDP. Further evaluation is necessary to deter-

[2]We thank G. Doran for performing the LDH assays and G. A. Currie for valuable advice.

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mine if local measures (eg, injection of steroids followed by ice) after infiltration will reduce the tissue damage.

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ANNOUNCEMENTS

1981 Schedule for Subspecialty Examination in Medical Oncology

Beginning in 1981 American Board of Internal Medicine subspecialty examinations will revert to the former fall date. The registration period is January 1–April 1, 1981. The examination date is November 10, 1981. The deadline for cancellation is October 1, 1981.

For application forms contact: American Board of Internal Medicine, 3624 Market St, Philadelphia, PA 19104 (215-243-1500).

Fourth Annual Vail Cancer Treatment Conference

Vail, Colorado, February 28–March 7, 1981.

The Beth Israel Hospital of Denver is sponsoring its Fourth Annual Vail Cancer Treatment Conference at the Kiandra-Talisman Lodge, Vail, Colorado, on February 28–March 7, 1981. Twenty-two hours of AMA Category I and AAFP Prescribed Credit are available. The general tuition is $260.00 ($180.00 for house officers). *For information regarding program content and housing, contact:* Beth Israel Conference Program, PO Box 11366, Denver, CO 80211 (303-629-5333 or 800-525-5810 [toll-free outside Colorado]).

Sixty-Third Annual Meeting of the American Radium Society

Phoenix, Arizona, March 4–8, 1981

The Sixty-Third Annual Meeting of the American Radium Society will be held at the Hyatt Regency Hotel in Phoenix, AZ on March 4–8, 1981. *For information please contact:* Mrs. Sally Polek, Office of the Secretariat, American Radium Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3181).

Symposium on Gastroenterology

Yosemite National Park, California, March 15–18, 1981

A Symposium on Gastroenterology will be held at the Ahwahnee Hotel in Yosemite National Park, California, on March 15–18, 1981. This is the second Yosemite Gastroenterology Symposium organized jointly by the Division of Gastroenterology and Extended Programs in Medical Education, University of California School of Medicine, San Francisco, and the Central California Committee for Gastroenterology. It is designed to provide the internist and family practitioner with an intensive review of gastrointestinal disorders, their diagnosis, and current therapy. Practical *management* information will be emphasized. In addition to didactic presentations, case discussions and concurrent seminars will provide the opportunity for effective participant/faculty interactions and thorough discussion of specific problems.

Half-day didactic sessions will focus on: 1) *the colon,* including constipation, diarrhea, bleeding, polyps and cancer, colitis, proctitis, and diverticulosis/diverticulitis; 2) *the liver and biliary tract,* including hepatitis, diagnostic tests, obstructive diseases of the biliary tree, biliary surgery, and endoscopic retrograms; and 3) *peptic diseases and the esophagus,* including reflux esophagitis, esophageal varices, cimitidine, surgery, and gastric ulcer-cancer.

This program meets the criteria for 16 hours in Category I of the AMA Physician's Recognition Award and the CMA Certification Program. This program is also approved for 16 elective hours by the American Academy of Family Physicians. *For further information, please contact:* Fresno Division, Extended Programs in Medical Education, c/o Area Health Education Center, 5110 E Clinton Way, Suite 201, Fresno CA 93727.

International Symposium on Surgical Treatment and Reconstruction for Breast Cancer

Madrid, Spain, March 20–21, 1981

An International Symposium on Surgical Treatment and Reconstruction for Breast Cancer will be held in Madrid, Spain, on March 20–21, 1981. *For further information, please contact:* Dr. R. de la Plaza, Red Cross Centre for Burns and Plastic Surgery, Calle Lisboa, 6, Madrid 8, Spain.

Second International Congress on Senology

Barcelona, Spain, May 25–29, 1981

The Second International Congress on Senology will be held at the Palacio de Congresos, in Barcelona, Spain, on May 25–29, 1981. *For information, contact:* Scientific Secretariat, Faculty of Medicine, Cátedra de Patologia Quirúrgica, Casanova, 143, Barcelona, 36, Spain *or* Technical Secretariat, Ultramar Express, SA, Diputación, 238, Barcelona, 7, Spain.

Fifth Asia Pacific Cancer Conference

Colombo, Sri Lanka, September 1–4, 1981

The Fifth Asia Pacific Cancer Conference will be held at the Bandaranaike Memorial International Conference Hall, Bauddhaloka Mawatha, in Colombo, Sri Lanka on September 1–4, 1981.

The theme of the conference is "malignancies of the gastrointestinal tract." *Papers:* Papers on the conference theme will be preferred. But papers on other aspects of cancer research (including epidemiology, etiology, pathology, experimental oncology, diagnosis, and treatment) and on cancer control activities will be accepted. Relevance of the subjects to the Asia Pacific region would be of added advantage. Proffered papers should not exceed 10 minutes.

Registration fees are as follows: $150.00 for delegates and $100.00 for accompanying persons before February 28, 1981; and $175.00 for delegates and $120.00 for accompanying persons after March 1, 1981. *Address of conference secretariat:* Secretary General, Conference Secretariat, c/o Sri Lanka Cancer Society, 37/25 Bullers Lane, Colombo 7, Sri Lanka.

Symposium on Hodgkin's Disease

San Francisco, California, September 9–12, 1981

The National Cancer Institute and the Cancer Clinical Investigation Review Committee are sponsoring a major symposium entitled "Contemporary Issues in Hodgkin's Disease: Biology, Staging, and Treatment" to be held at the San Francisco Hilton, San Francisco, California, on September 9–12, 1981. An outstanding international faculty has been organized to review recent developments of importance with emphasis placed on controversial issues in clinical management. Proffered papers will also be selected for presentation. Abstract forms are now available; the deadline for submission of abstracts is March 1, 1981. Those wishing to attend must preregister and will be accommodated on a first-come, first-served basis. There will be a registration fee of $50.00. *For abstract forms or further information, please contact:* Lili Zubar, Box 277 University of Minnesota Hospitals, 420 Delaware St, SE, Minneapolis, MN 55455 (612-373-4309).

Sixth Meeting of the European Association for Cancer Research

Budapest, Hungary, October 12–15, 1981

The Sixth Meeting of the European Association for Cancer Research will be held in Budapest on October 12–15, 1981. The meeting will be devoted mainly to the presentation of experimental and clinical data on two topics: 1) metastasis and 2) tumor-associated markers in diagnosis and therapy.

For additional information, contact: **Dr. A. Jeney, I. Institute of Pathology and Experimental Cancer Research, Semmelweis Medical University, Üllöi ut 26, H-1085 Budapest, Hungary.**

Union Internationale Contre le Cancer Conference on Clinical Oncology

Lausanne, Switzerland, October 29–31, 1981

The Union Internationale Contre le Cancer Conference on Clinical Oncology will be held in Lausanne, Switzerland, on October 29–31, 1981. The conference is organized by the International Union Against Cancer in conjunction with the Seventh Meeting of the European Society of Medical Oncology. The conference is hosted by the Swiss Cancer League and is co-sponsored by the European Organizatin for Research on Treatment of Cancer, the European Association for Cancer Research, and the Organization of European Cancer Institutes.

This multidisciplinary conference on cancer treatment is a major innovation within the International Union Against Cancer. It is open to all physicians and is designed to provide a forum for presentation of original research in all clinical aspects of multidisciplinary treatment of cancer patients. Internationally renowned clinical oncologists will be invited as speakers and chairmen. Parallel sessions at which proffered papers are presented will be organized during the first part of each half day. Ample time will be provided for discussions. Major review lectures will be presented in plenary sessions during the second part of each half day by the chairmen of the proffered paper sessions. Poster sessions will be organized throughout the conference.

Further information can be obtained from: **The Conference Secretariat, PO Box 248, CH 1000, Lausanne 6, Switzerland.**

Symposium on Herpesviruses as Oncogenic Agents

Chapel Hill, North Carolina, April 2–3, 1981

The Cancer Research Center and The Department of Bacteriology and Immunology at the University of North Carolina School of Medicine at Chapel Hill are sponsoring a symposium on Herpesviruses as Oncogenic Agents on April 2–3, 1981. The symposium will focus on the role of viruses, particularly herpesviruses, in oncogenic transformation. Prominent virologists from throughtout the USA and one from abroad will present their most recent findings and ideas regarding this topic. The current thinking in herpesvirus oncogenesis will be discussed as part of the general problem of viral oncogenesis. The meeting will include a consideration of the control of herpesvirus infections by chemotherapy. The symposium will provide eleven hours of credit in continuing medical education in Category I AMA-PRA. ***For further information contact:*** **Dr. John Newbold, Cancer Research Center, Box 30, MacNider Bldg, Chapel Hill, NC 27514 (919-966-3036).**

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